Analysis of Genomic and Proteomic Signals Using Signal Processing and Soft Computing Techniques

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Analysis of Genomic and Proteomic Signals Using Signal Processing and Soft Computing Techniques

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dedicated to my parents with love...

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Certificate

This is to certify that the dissertation entitled ***"Analysis of Genomic and Proteomic Signals Using Signal Processing and Soft Computing Techniques"*** by ***Sitanshu Sekhar Sahu***, submitted to the National Institute of Technology, Rourkela for the degree of Doctor of Philosophy, is a record of an original research work carried out by him in the department of Electronics and Communication Engineering under my supervision. I believe that the thesis fulfills part of the requirements for the award of degree of Doctor of Philosophy. Neither this dissertation nor any part of it has been submitted for any degree or academic award elsewhere.

Ganapati Panda

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Abstract

Bioinformatics is a data rich field which provides unique opportunities to use computational techniques to understand and organize information associated with biomolecules such as DNA, RNA, and Proteins. It involves in-depth study in the areas of genomics and proteomics and requires techniques from computer science, statistics and engineering to identify, model, extract features and to process data for analysis and interpretation of results in a biologically meaningful manner. In engineering methods the signal processing techniques such as transformation, filtering, pattern analysis and soft-computing techniques like multi layer perceptron (MLP) and radial basis function neural network (RBFNN) play vital role to effectively resolve many challenging issues associated with genomics and proteomics.

In this dissertation, a sincere attempt has been made to investigate on some challenging problems of bioinformatics by employing some efficient signal and soft computing methods. Some of the specific issues, which have been attempted are protein coding region identification in DNA sequence, hot spot identification in protein, prediction of protein structural class and classification of microarray gene expression data. The dissertation presents some novel methods to measure and to extract features from the genomic sequences using time-frequency analysis and machine intelligence techniques.

The problems investigated and the contribution made in the thesis are presented here in a concise manner. The S-transform, a powerful time-frequency representation technique, possesses superior property over the wavelet transform and short time Fourier transform as the exponential function is fixed with respect to time axis while the localizing scalable Gaussian window dilates and translates. The S-transform uses an analysis window whose width is decreasing with frequency providing a frequency dependent resolution. The invertible property of S-transform makes it suitable for time-band filtering application. Gene prediction and protein coding region identification have been always a challenging task in computational biology, especially in eukaryote genomes due to its complex structure. This issue is resolved

using a S-transform based time-band filtering approach by localizing the period-3 property present in the DNA sequence which forms the basis for the identification. Similarly, hot spot identification in protein is a burning issue in protein science due to its importance in binding and interaction between proteins. A novel S-transform based time-frequency filtering approach is proposed for efficient identification of the hot spots. Prediction of structural class of protein has been a challenging problem in bioinformatics. A novel feature representation scheme is proposed to efficiently represent the protein, thereby improves the prediction accuracy. The high dimension and low sample size of microarray data lead to curse of dimensionality problem which affects the classification performance. In this dissertation an efficient hybrid feature extraction method is proposed to overcome the dimensionality issue and a RBFNN is introduced to efficiently classify the microarray samples.

In essence, this dissertation employs some latest signal and soft-computing tools for obtaining the patterns present in the DNA and protein sequences as well as to develop efficient feature extraction method for achieving better classification.

***Keywords*: Gene, Exon, Protein, Hot spot, Microarray, Time-frequency analysis, S-transform, DCT, AmPseAAC, AR Modeling, F-score**

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List of Acronyms

DNA Deoxy Ribo Nucleic Acid

RNA Ribo Nucleic Acid

FT Fourier Transform

DFT Discrete Fourier Transform

IDFT Inverse Discrete Fourier Transform

FFT Fast Fourier Transform

DCT Discrete Cosine Transform

STFT Short Time Fourier Transform

WT Wavelet Transform

CWT Continuous Wavelet Transform

MLP Multi Layer Perceptron

RBFNN Radial Basis Function Neural Network

ROC Receiver Operating Characteristics

RRM Resonant Recognition Model

$\mathbf{S}_n$ Sensitivity

$\mathbf{S}_p$ Specificity

PPV Positive Predictive Value

NPV Negative Predictive Value

AmPseAAC Amphiphilic Pseudo Amino Acid Composition

AAC Amino Acid Acid Composition

AR Auto Regressive

ANN Artificial Neural Network

DSP Digital Signal Processing

TBP Three Base Periodicity

EIIP Electron Ion Interaction Potential

TFA Time Frequency Analysis

TFR Time Frequency Representation

AUC Area Under the Curve

ASM Alanine Scanning Mutagenesis

ASA Solvent Accessible Surface Area

SVM Support Vector Machine

SNR Signal to Noise Ratio

FGF Fibroblast Growth Factor

PDB Protein Data Bank

LOOCV Leave One Out Cross Validation

LDA Linear Discriminant Analysis

Chapter 1

Introduction

Chapter 1

Introduction

Bioinformatics as a discipline has become an essential part of discoveries in molecular biology. It was created based on the strong need to make sense of the massive amount of biological information made available by the Human genome project and similar initiatives of many public and private organizations across the world. It makes the use of computational techniques to understand and organize information associated with biomolecules. These biomolecules include genetic materials such as nucleic acids, deoxy ribo nucleic acid (DNA), ribo nucleic acid (RNA) and proteins, which give rise to two basic areas of research: genomics and proteomics. This requires efficient techniques and methodologies to organize, analyze, and interpret the results in a biologically meaningful manner. The high variability in the data acquisition process, the huge dimension of the data space and the high complexity of genetic signals call for sophisticated mathematical modeling, data processing and information extraction methods. It involves the use of techniques from computer science, statistics and engineering to solve various biological problems. The digital nature of genomic information makes it suitable for the application of signal processing concepts, tools and techniques to better analyze and understand the characteristics of DNA, RNA, proteins and their interactions. Signal processing techniques like various transformation methods, filtering and pattern classification can be effectively used for the analysis of genomic and proteomic signals. Prediction of genes, protein structure, and protein function greatly utilize pattern recognition

techniques, in which machine learning play a central role. Hence application of signal processing and soft computing techniques have potential future to facilitate better understanding of the processes, functions and structures associated with bioinformatics problems.

1.1 Background and scope of the thesis

Genomic sequence, structure and function analysis of various organisms have been a challenging problem in bioinformatics [1].The exponential growth of the repository of genomic sequences through many scientific and biological communities has put a major thrust into the genome research.Basically genes are repositories for protein coding information and proteins in turn are responsible for most of the important biological functions in all cells.The determination of patterns in DNA sequences is useful for many important biological problems such as identifying new genes, pathogenic islands and phylogenetic relationships among organisms. Hence accurate prediction of genes has always been a challenging task for computational biologists, especially in eukaryote genomes due to its complex structure and the presence of background noise in the sequence. However it has been found that the bases in the protein coding regions exhibit a period-3 property due to the codon bias involved in the translation process which has been used as a good indicator of exon location. Many methods have been applied for the identification of coding regions which are based on the Fourier spectral content, spectral characteristics, correlation of structure of DNA sequences and digital filtering [2] [3] [43]. Still it needs an improvement in the prediction accuracy and also in the computational complexity of the algorithm.

The biological mechanisms of living organisms like metabolism, gene regulatory and interaction pathways have put numerous challenges to modern bimolecular research. In particular, structural identification and characterization of protein-protein interactions are crucial in protein science due to their complexity [83] [80]. The protein-protein interactions provide a base to identify and analyze

the drugs, molecular medicines, etc. These interactions are very selective in nature. Proteins interact with the target molecules at specific sites known as active sites and certain residues that operate as key in binding and recognition are termed as hot spots. Several structure and sequence based computational methods have been proposed to identify the hotpots in proteins. Basically these are feature based classification models which uses some characteristics of protein for the prediction. Recently a signal processing technique, digital filtering [83] has been applied for this purpose, which fails to uniquely detect the characteristic frequencies relevant to the hot spot. Hence there is a need to apply the computational tools and techniques to completely understand the mechanism behind the interactions.

Prediction of physical structures and subsequent separation into characteristics groups is also important for analyzing the functional influences of biologically vital proteins. In the post genomic era the study of sequence to structure relationship and functional annotation plays an important role in molecular biology [89] [90]. In this context, the protein fold prediction is one of the major challenges in protein science. The structural class has become one of the most important features for characterizing the overall folding type of a protein and has played an important role in rational drug design, pharmacology and many other applications. Hence there exists a critical challenge to develop automated methods for fast and accurate determination of the structures of proteins. The problem of predicting protein structural class mainly focused on effective representation of protein sequences and then development of the powerful classification algorithms to efficiently predict the class attribute. Several in-silico prediction techniques with many amino acid indices and features have been used for the class prediction issue. Still the accuracy in prediction needs to be improved which demand an efficient feature representation of protein sequences.

Recent advances in microarray technology have accrued a huge amount of gene expression profiles of tissue samples at relatively low cost which facilitates scientists and researchers to characterize complex biological problems. Microarray technology has been used as a basis to unravel the interrelationships among genes such as

clustering of genes, temporal pattern of expressions, understanding the mechanism of disease at molecular level and defining of drug targets [134] [137]. Generally the microarray experiments produce large datasets having expression levels of thousands of genes with a very few numbers (order of hundreds) of samples. Thus, it creates a problem of "Curse of dimensionality". Due to this high dimension, the accuracy of the classifier decreases as it attains the risk of overfitting. Several dimension reduction methods have been applied in conjunction with many artificial intelligence techniques to efficiently analyze the microarray data. Hence there is a need to develop efficient feature extraction and selection methods for the classification and clustering of microarray gene expression data.

1.2 Motivation

A lot of research ideas have gone into the development of predictive models and feature extraction methods based on a range of signal processing and artificial intelligence techniques over the past few decades to analyze various problems associated with bioinformatics. There are some significant issues (as mentioned below) in various bioinformatics problems which need to be addressed and resolved.

1. There are several existing literatures available in protein coding region identification. However, the prediction accuracy of the existing techniques suffers due to the presence of background noise in the DNA sequence.

2. The hotspot identification problem has been handled by the conventional digital filtering technique. However, this approach fails to retrieve the characteristic frequency component at localized regions in protein sequence based on time-frequency localization.

3. One of the key issues is the feature representation which affect the performance accuracy of the protein structural class prediction. The existing research works investigated the problem by incorporating the sequence order and length information with the composition of amino acids in the protein sequence.

However, the classification accuracy needs to be further improved for better prediction of structural class.

4. Microarray data classification is essentially a data mining problem in bioinformatics and thus needs efficient feature selection and extraction to improve the classification accuracy. Hence standard and organized feature selection process needs to be investigated for further enhancing the accuracy measure.

5. Measuring and processing the genomic and proteomic signal in time domain and frequency domain alone do not provide the complete information regarding the structure, sequence and pattern of the molecules. Hence, a joint time-frequency analysis is needed to provide a better understanding of the hidden artifacts in genomic signals. Time-frequency representations describe signals in terms of their joint time and frequency contents.

The above issues embodied in this dissertation have motivated us to carry out research work by developing potential signal processing and soft computing based techniques.

1.3 Objective of the dissertation

The objective of present research work is to contribute towards furnishing novel signal processing measures and features for analysis of DNA sequences, protein and high throughput microarray samples. In summary, the main objectives of this research work are:

- To formulate a close relationship between genomic sequence analysis issues and signal processing theories.

- To select suitable signal processing tools either for calculation of measure or for extraction of features from mapped genomic sequences and gene expression profiles.

- To introduce a novel time-frequency analysis technique to identify or predict the patterns present in the genomic or proteomic signals.
- To devise a new feature representation for prediction of the protein structural classes.
- To propose a hybrid feature extraction method for efficient classification of the cancer microarray gene expression profiles.
- To introduce simple and efficient machine learning techniques for the pattern identification problems.
- To deal with huge genomic and microarray data in an efficient and effective manner.

A sincere attempt has been made to address all these issues in this dissertation.

1.4 Dissertation Outline

The outline of the dissertation is as follows:

Chapter 1

This chapter contains an introduction to the bioinformatics problems undertaken for the analysis, the motivation and the objectives of the research work. It also contains the chapterwise contribution made in the dissertation.

Chapter 2

A brief outline of the signal processing methods and soft computing techniques employed for identification and classification purpose are presented in this chapter. This includes the conventional transformation techniques (such as discrete Fourier transform (DFT), discrete cosine transform (DCT)) and the time-frequency representations (such as the short time Fourier transform (STFT), the wavelet transform (WT) and the S-transform). This Chapter also reviews the existing machine learning techniques, such as the MLP and the RBF networks, which have been used in subsequent investigation.

Chapter 3

In this chapter, a novel time-frequency filtering scheme has been proposed for the identification of protein coding regions in DNA sequence. The motivation behind this investigation is to improve the accuracy of prediction of the coding regions. First, the spectrum of the DNA sequence is computed to localize the period-3 component in time-frequency plane, which forms the basis of the identification. Then, this pattern is filtered out using a mask in the time-frequency domain, thereby producing peaks in the energy sequence wherever the coding regions are present. The results obtained using the proposed approach are compared with those obtained by DFT and anti-notch filter methods through the ROC curve analysis and statistical measures such as sensitivity, specificity and average accuracy.

Chapter 4

A novel S-transform based filtering approach is proposed in this chapter to identify the hot spots in proteins. It is a sequence based approach which uses the sequence information rather than structural information to detect the hot spots based on the resonant recognition model (RRM). The RRM correlates the biological functioning of the protein to the characteristic frequencies which is obtained through the consensus spectrum of the functional group. The hot spots which are relevant to the functioning of the protein have been identified by localizing the characteristic frequency along the protein sequence. First, the spectrum of the protein sequence is obtained to show the energy distribution of the frequencies in the time-frequency domain. Then, a time and band limited filter is used on the time-frequency spectrum to extract the characteristic frequency. The energy of the filtered sequence produces peaks corresponding to the hot spots. The performance of the proposed method is compared with the corresponding results obtained by existing computational methods, such as digital filtering method, KFC server, Hotsprint, ISIS and HotPOINT in terms of sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and average accuracy.

Chapter 5

This chapter presents a new feature representation scheme based on the Chou's pseudo amino acid composition for efficient prediction of protein structural class. It has suitably embedded the amino acid composition information, the amphiphilic correlation factors and the spectral characteristics of the protein to form a new pseudo amino acid feature vector (DCTAmPseAAC). An exhaustive simulation study is carried out on the standards 204, 277 and 498 datasets to show the efficiency of the new feature representation. A simple radial basis function neural network is introduced to predict the structural class which provides better results as compared to other feature representation and computational methods.

Chapter 6

An efficient hybrid feature extraction method is presented in this chapter to combat the curse of dimensionality problem occurring in the classification analysis of microarray gene expression data. First, the F-score method is applied on the gene space to select the discriminative features from the microarray samples. Then, autoregressive modeling (AR) is employed on the reduced feature subset to model and capture the global characteristics of the genes among the samples. A low complexity machine learning technique, the RBFNN, is introduced to efficiently classify the cancer microarray samples. The performance of the proposed method is assessed and compared with the existing methods using standard datasets.

Chapter 7

The overall conclusion of the investigation is reported in this chapter. This chapter also contains the details of further research work that can be done in the same or the related field.

1.5 Conclusion

This chapter provides a brief introduction to bioinformatics, its present day importance and its associated problems. It also systematically outlines the scope, the motivation which resulted in the investigation and the objectives of the thesis. A

concise presentation of research work carried out in each chapter and the contribution made have also been dealt. In essence this chapter provides a complete overview of the total thesis in a condensed manner.

Chapter 2

Signal Processing and Soft-Computing Techniques Employed in the Investigation

Chapter 2

Signal Processing and Soft-Computing Techniques Employed in the Investigation

2.1 Introduction

The large scale and rapidly growing biological databases generated by the advanced technologies in the form of millions of DNA, protein sequences and microarray data, provide information for revealing the molecular functions and structures. In order to interpret these genomic information in a meaningful manner, we require fast, efficient and intelligent techniques from science and engineering. This chapter presents a brief review of the signal processing and soft-computing techniques used for solving some challenging problems in bioinformatics.

2.2 Signal processing techniques used in the analysis

A brief introduction of the signal processing tools and techniques used for the analysis of genomic sequences is provided in this section.

2.2.1 Discrete Fourier transform

In many real world applications, the signals represented in time domain, at times, is unable to infer the hidden information and patterns in the signal. Therefore, it is necessary to represent the signal in some alternate domains where the internal characteristics of the signal can be reflected in a better way. The Fourier transform (FT) provides such a representation by transforming a signal from time domain into frequency domain. The Fourier transform is an invertible integral transform that expresses a function in terms of sinusoidal basis functions, i.e. as a sum or integral of sinusoidal functions of different frequencies [4].

The Fourier transform $X(f)$ of a signal $x(t)$ is defined as

$$X(f) = \int_{-\infty}^{\infty} x(t) e^{-j2\pi ft} dt \tag{2.1}$$

and its inverse relationship is given by

$$x(t) = \int_{-\infty}^{\infty} X(f) e^{j2\pi ft} dt \tag{2.2}$$

The discrete version of the Fourier transform is called the discrete Fourier transform (DFT). This is used when both the time and the frequency variables are discrete. The DFT of a discrete time signal $x(n)$ of length N can be viewed as a uniformly sampled version of $X(f)$ at frequencies $f_k = \frac{k}{N}$, for $k = 0, 1, \cdots, N-1$. The period of the signal is $\frac{N}{k}$. The DFT of the signal $x(n)$ is defined as

$$X\left(\frac{k}{N}\right) = \frac{1}{N} \sum_{n=0}^{N-1} x(n) e^{-\frac{j2\pi nk}{N}} \tag{2.3}$$

Hence the inverse DFT (IDFT) is defined as

$$x(n) = \sum_{k=0}^{N-1} X\left(\frac{k}{N}\right) e^{\frac{j2\pi nk}{N}} \tag{2.4}$$

The discrete Fourier transform is one of the most common spectral analysis technique and has been used in various fields such as image analysis, filtering, pattern analysis, feature extraction in various areas in engineering, chemistry, biology, etc [2] [3]

[4]. There exist an efficient algorithm, known as fast Fourier transform (FFT) to reduce the computational complexity in computing the DFT and its inverse. Direct computation of the DFT coefficients (N-point which is a power of 2) requires $O(N^2)$ operations, whereas the FFT algorithm can compute the same in only $O(Nlog_2N)$ operations. The DFT $X(k)$ of a signal $x(n)$ produces the frequency components

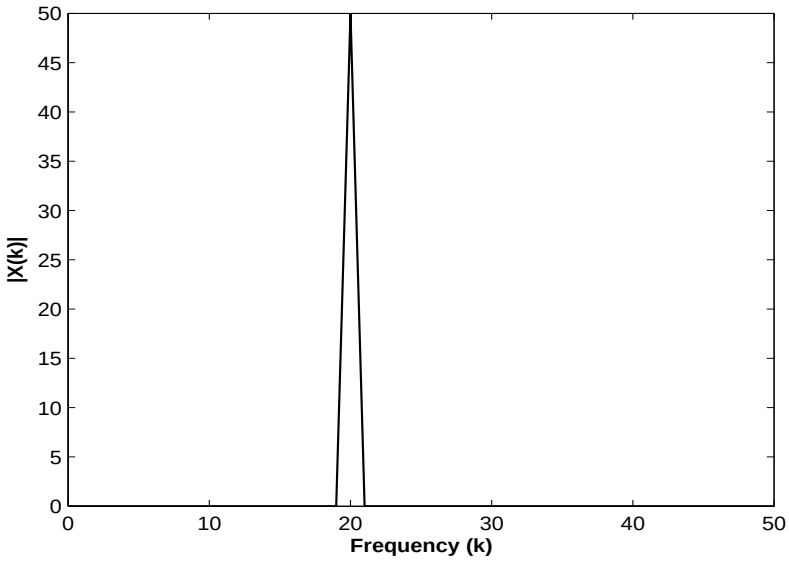


Figure 2.1: The magnitude plot of the DFT of a periodic signal with a period $T = 5$.

present in the signal. For an illustration, let us consider a signal of length 100, having a period-5 component. The DFT of the signal is shown in Fig. 2.1 . The period 5 component is clearly shown by a peak in the plot at frequency $k = \frac{100}{5} = 20$. This property of DFT can be used to identify the periodicities present in the signal. Further details of the DFT and its properties can be found in the Ref. [4].

2.2.2 Discrete cosine transform

The discrete cosine transform (DCT) is a very well studied technique and has been successfully applied to variety of applications such as data compression, feature extraction and classification [6] [7]. The DCT is a real-valued and quasi-orthogonal transformation, that preserves the norms and angles of the vectors. It represents

a finite sequence of data points in terms of sum of cosine functions oscillating at different frequencies [5]. The DCT $G(k)$ of a signal $x(n)$ is defined as

$$G(k) = a(k) \sum_{n=0}^{L-1} x(n) cos \left[\frac{(2n+1)k\pi}{2L} \right], k = 0, 1, 2, \cdots\cdots, L-1 \qquad (2.5)$$

where $$a(k) = \begin{cases} \sqrt{\frac{1}{L}}, k = 0 \\ \sqrt{\frac{2}{L}}, k \neq 0 \end{cases}$$

where $G(0)$ represents the average value of the signal and is called the DC or constant component and the remaining are called the time varying or harmonic of the sequence.

In particular, a DCT is a Fourier-related transform similar to the DFT, but uses only real numbers with even symmetry. Therefore, it involves lower computational complexities than the DFT. In DFT, the time signal is truncated and is assumed periodic. Hence, discontinuity is introduced in time domain and some corresponding artifacts are introduced in the frequency domain. But, in DCT, since even symmetry is assumed while truncating the time signal, no discontinuity and related artifacts are present. The DCT leads to uncorrelated transform coefficients, which can be processed independently, thereby reduces the redundancy present in the signal. It also exhibits excellent energy compaction for highly correlated images.

2.2.3 Time-frequency analysis

Most of the signals in nature such as in geophysics, biology, environment are non stationary and time varying. Energy distributions of non stationary signals can not be analyzed using the classical power spectrum methods based on Fourier transform. The Fourier transform of a signal gives only information about the frequency contents of the signal. However, it does not give any explicit indication about when a frequency component is present, since the value of the Fourier transform is evaluated by averaging the contributions from all time. For non stationary time series, the spectral content changes with time and hence, the time averaged amplitude spectrum computed using Fourier transform is inadequate to track the changes.

No information can be induced from the Fourier amplitude spectrum on when a particular frequency component exists in a signal. Actually, the time information of the spectral elements are hidden in the phase spectrum of the signal. Again, if a particular frequency signal exists for a very small duration in a long time series, that frequency can not be noticed in the amplitude spectrum. This restriction gives rise to a new era of spectrum analysis known as time-frequency analysis. These phenomena demands more efficient way of signal analysis to locally and simultaneously characterize the signal in both time and frequency domains [8] [9]. The main idea of time frequency distribution is to devise a two dimensional function of both time and frequency, which will describe the spectral changes in the signal simultaneously in time and frequency [10]. The most important and widely used time-frequency representations for spectrum analysis in various fields are: short time-Fourier transform (STFT), wavelet transform (WT) and the S-transform. These techniques are described in detail below.

Short time Fourier transform

The STFT is a Fourier related transform used to determine the sinusoidal frequency and phase content of local sections of a signal as it changes over time [11]. It localizes the frequency components in time by sliding a window along the signal and computing the Fourier transform of the windowed signal. The STFT spectrum (S) of a signal $x(t)$ is defined as

$$S_{STFT}(\tau, f) = \int_{-\infty}^{\infty} x(t)w(t-\tau)e^{-j2\pi ft}dt \tag{2.6}$$

where $w(t)$ is the window function. Since, these basis functions are translated and modulated versions of the window, they are centered at different time locations in the time-frequency plane. STFT has a fixed time and frequency resolution. The resolution depends on the width of the window function. Frequency resolution is proportional to the bandwidth of the windowing function while time resolution is proportional to its length. Thus a short window is needed for good time resolution and a wider window offers good frequency resolution.

Wavelet transform

To partially overcome the problem of fixed resolution with STFT, wavelet transform (WT) is evolved which introduces a basis function (wavelet) whose width varies with scale to provide multi-resolution analysis [9] [12]. The wavelet transform of a signal $x(t)$ is defined as

$$S_{WT}(a,b) = \int_{-\infty}^{\infty} x(t)\psi^*_{a,b}(t)dt \tag{2.7}$$

where $\psi^*(t)$ is the specific mother wavelet, a is the dilation parameter and b is the translation parameter. Hence, the mother wavelet is defined as

$$\psi^*_{a,b}(t) = \frac{1}{\sqrt{a}}\psi\left(\frac{t-b}{a}\right) \tag{2.8}$$

The wavelet transform is computed by the inner product of the signal, the dilations and translations version of the mother wavelet. WT is represented as a time scale plot, where scale is the inverse of frequency. To analyze the low frequency components in the signal, the analyzing wavelet is dilated in time and compressed in frequency. To analyze the high frequency components, the analyzing wavelet is dilated in frequency and compressed in time. This property of WT makes it very suitable for analysis of signals of high frequency with short duration and low frequency with long duration. The interpretation of the time scale representations produced by the wavelet transform require the knowledge of the type of the mother wavelet used for the analysis. Also the wavelet transform does not retain the absolute phase information and the visual analysis of the time-scale plots that are produced by the WT is intricate to interpret.

S-transform

The S-Transform is the hybrid of short time Fourier transform and wavelet transform. It uses a Gaussian window whose width scales inversely and height scales directly to provide a frequency dependent resolution while maintaining a direct relationship with Fourier spectrum. The S-transform is a time-frequency analysis technique proposed by Stockwell *et al.* [13], which combines the properties of the

short time Fourier transform and the wavelet transform. The standard S-Transform of a signal $x(t)$ is defined as

$$S(\tau, f) = \int_{-\infty}^{\infty} x(t) w(\tau - t, f) e^{-j2\pi ft} dt \tag{2.9}$$

The window function used in the S-transform is a scalable Gaussian function given as

$$w(t, \sigma) = \frac{1}{\sigma(f)\sqrt{2}\pi} e^{-\frac{t^2}{2\sigma^2(f)}} \tag{2.10}$$

and the width of the window varies inversely with frequency as

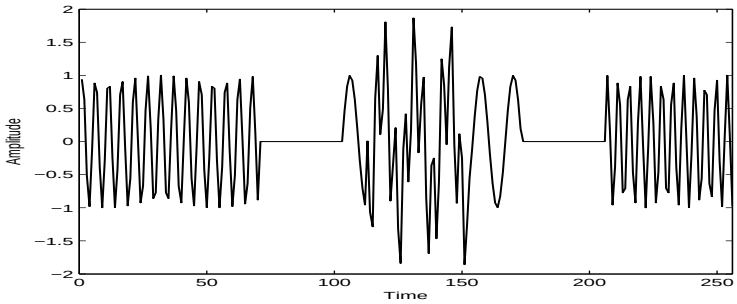
$$\sigma(f) = \frac{1}{|f|} \tag{2.11}$$

Combining Eqs. (2.10) and (2.11) gives

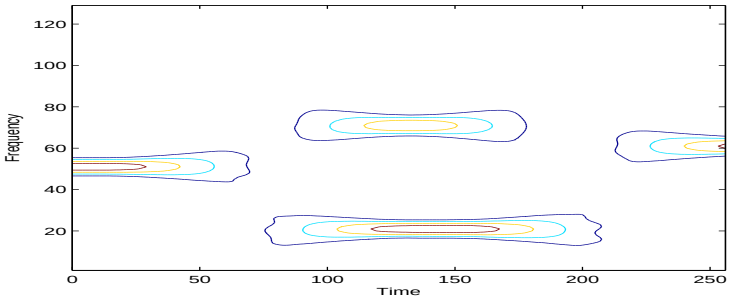
$$S(\tau, f) = \int_{-\infty}^{\infty} x(t) \left\{ \frac{|f|}{\sqrt{2}\pi} e^{-\frac{(\tau - t)^2 f^2}{2}} e^{-j2\pi ft} \right\} dt \tag{2.12}$$

The advantage of the S-transform over the short time Fourier transform is that the window width (σ) is a function of frequency (f) rather than a fixed one as in STFT and thereby provides multiresolution analysis. In contrast to wavelet analysis, the S-Transform wavelet is divided into two parts as shown within the braces of Eq. (2.12). One is the slowly varying envelope (the Gaussian window) which localizes the time and the other is the oscillatory exponential kernel which selects the frequency being localized. It is the time localizing Gaussian that is translated while keeping the oscillatory exponential kernel stationary, which is different from the wavelet kernel. As the oscillatory exponential kernel is not translating, it localizes the real and the imaginary components of the spectrum independently, thus localizing the phase as well as the amplitude spectrum. Therefore, it retains the absolute phase of the signal which is not provided by wavelet transform.

Let us analyse a synthetic time series in order to show the localizing property of the time-frequency representations. It consists of four different frequencies 20 Hz, 50 Hz, 60 Hz and 70 Hz present at different locations in the time series. The 20 Hz signal presents during 103-173 samples, the 50 Hz signal presents at 1-70 samples,



(a)



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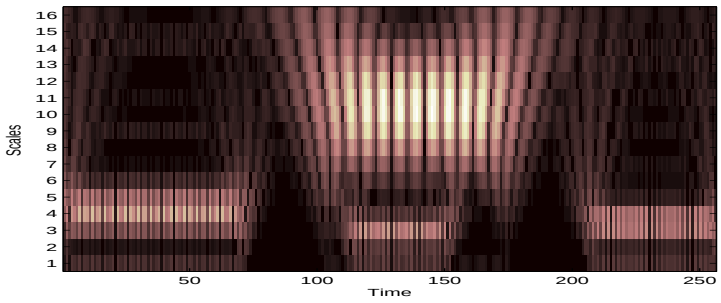

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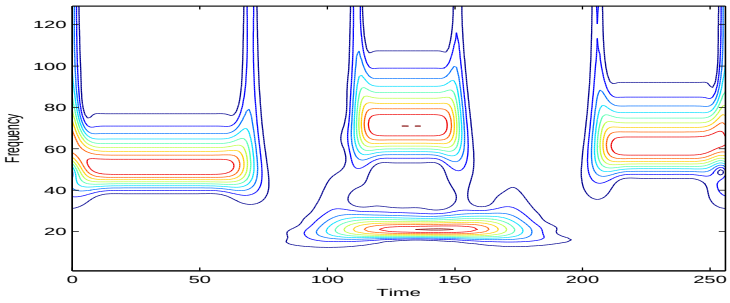


(d)

Figure 2.2: Comparison of the power spectra obtained by STFT, WT and S-transform, (a) The synthetic time series (composition of four frequency signals: 20Hz, 50Hz, 60 Hz and 70 Hz) (b) Spectral plot of the time series using STFT (c) Spectral plot of the time series using continuous wavelet transform (CWT) (d) Spectral plot of the time series using S-transform

the 60Hz signal mixed with 20Hz component during 112-152 samples and the 70Hz component presents at 206-256 samples. The spectrum of the time series is computed using STFT, WT and S-transform and is shown in Fig.2.2. The STFT provides a poor detection capability in time and frequency direction as it smears in both the direction. The wavelet transform provides a messy information about the frequency components. The S-transform shows better localization capability, but smears in frequency direction in higher frequencies. Due to its better time-frequency detection and phase containt capability, it has been popularly used in geophysics, electrical and biomedical engineering [14, 17]. In this dissertation, this has been extensively studied in identification of patterns in biological signals.

In Eq. (2.12) the S-transform window satisfies the condition

$$\int_{-\infty}^{\infty} w(t,f)dt = 1 \tag{2.13}$$

Therefore, averaging the $S(\tau, f)$ over all values of t yields $X(f)$, the Fourier transform of $x(t)$.

$$\int_{-\infty}^{\infty} S(\tau,f)d\tau = X(f) \tag{2.14}$$

Hence, the original signal can be recovered by using the inverse Fourier transform of $X(f)$.

$$x(t) = \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} S(\tau,f)d\tau \right\} e^{j2\pi ft} df \tag{2.15}$$

Thus, it provides a direct link between the S-transform and the Fourier transform. Due to the invertibility property of the S-transform, it can be suitably used for time-frequency filtering [16, 17].

2.3 Soft computing techniques used in the analysis

Soft Computing is an emerging field that imitate human intelligence with the goal of creating tools provided with some human-like capabilities such as learning, reasoning, and decision making. Soft computing techniques have wide applications

in engineering and science due to their strong learning and cognitive ability and good tolerance of uncertainty, imprecision, partial truth, and approximation. Learning is the foundational discipline of the soft computing paradigm. Just as to learn in animals and humans, machines/computers also can be made intelligent by learning which leads to a new era of study known as machine learning.

Machine learning

Machine learning is derived from the efforts of psychologists, to make more precise their theories of animal and human learning through computational models. It refers to a system capable of acquiring and integrating the knowledge automatically. The capability of the systems to learn from experience, training, analytical observation, results in a system that can continuously self-improve and thereby exhibit efficiency and effectiveness. A major focus of machine learning research is to automatically learn to recognize complex patterns and make intelligent decisions based on data samples. The difficulty is that the set of all possible behavior given all possible inputs is too large to be covered by the set of observed samples (training data). Hence, the learner must generalize from the given samples, so as to be able to produce a faithful output in new observations. Traditionally, learning in machine intelligent has been studied either in the unsupervised paradigm where all the data are unlabeled or in the supervised paradigm where all the data are labeled.

Supervised learning

Supervised learning is the machine learning task of inferring a function from supervised training data. The training data consist of a set of training examples. Each example is a pair consisting of an input object (training data) and a desired output value (the supervisory signal). A supervised learning algorithm analyzes the training data and produces an inferred function, which is called a classifier or a regression function. The inferred function should predict the correct output value for any valid input object. This requires the learning algorithm to generalize from the training data to unseen situations in a reasonable way.

Unsupervised learning

Unsupervised learning refers to the problem finding hidden structure in unlabeled data. Since the observations given to the learner are unlabeled, there is no error or reward signal to evaluate a potential solution. The unsupervised learning includes clustering, blind source separation, outlier detection etc.

Another kind of machine learning is reinforcement learning.The training information provided to the learning system by the environment(external trainer)is in the form of a scalar reinforcement signal that constitutes a measure of how well the system operates. The learner is not told which actions to take, but rather discover which actions yield the best reward, by trying each action in turn.

The components of soft computing includes Artificial neural networks, Fuzzy logic and evolutionary computing algorithms. In this dissertation work, the artificial neural networks such as multi layer perceptron (MLP) and radial basis function network (RBF) with supervised learning have been used and are discussed below.

2.3.1 Artificial neural network

An artificial neural network (ANN) is an information processing system that tries to simulate biological neural networks i.e the nervous system in brain [18] [19]. Due to its nonlinear processing, learning capability and massively parallel distributed structure, ANN's have become a powerful tool for many complex applications including functional approximation, nonlinear system identification, control, pattern classification and optimization [20]- [23]. McCulloch and Pitts first developed the neural networks in 1943 for different computing machines. The ANN is capable of performing nonlinear mapping between the input and output space due to its large parallel interconnection between different layers and the nonlinear processing characteristics. An artificial neuron basically consists of a computing element that performs the weighted sum of the input signal and the connecting weight. The sum is added with the bias or threshold and the resultant signal is then passed through a nonlinear function. Each neuron is associated with three parameters whose learning

can be adjusted. These are the connecting weights, the bias and the slope of the nonlinear function. For the structural point of view a neural network may be single layer or it may be multilayer. In multilayer structure, there is one or many artificial neurons in each layer and for a practical case there may be a number of layers. Each neuron of the one layer is connected to each and every neuron of the next layer.

Single neuron structure

The operation in a single neuron involves the computation of the weighted sum of inputs and threshold. The resultant signal is then passed through a nonlinear activation function. This is also called as a perceptron, which is built around a nonlinear neuron. The basic structure of a single neuron is shown in Fig. 2.3. The output associated with the neuron is computed as

$$y(k) = f\left[\sum_{j=1}^{N} W_j(k)X_j(k) + b(k)\right] \tag{2.16}$$

where $X_i,\ \ i = 1, 2, \cdots, N$ are inputs to the neuron, w_j is the synoptic weights of the jth input, b_k is the bias, N is the total number of inputs given to the neuron and $f(.)$ is the nonlinear activation function. The activation functions generally used in neural computation are discussed below.

Activation functions

Log-sigmoid function

This function takes the input and squashes the output into the range of 0 to 1. This function is represented as

$$f(x) = \frac{1}{1 + e^{-x}} \tag{2.17}$$

Hyperbolic tangent sigmoid

This function is defined as

$$f(x) = tanh(x) = \frac{e^{x} - e^{-x}}{e^{x} + e^{-x}} \tag{2.18}$$

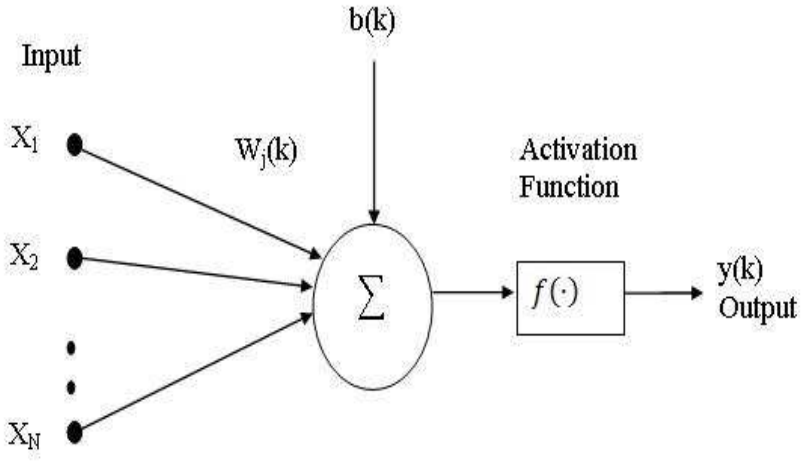


Figure 2.3: The structure of a single neuron

Signum function

This activation function is represented as

$$f(x) = \begin{cases} 1, \ if \ x > 0 \\ 0, \ if \ x = 0 \\ -1, \ if \ x < 0 \end{cases} \tag{2.19}$$

Threshold function

This function is given by the expression

$$f(x) = \begin{cases} 1, \ for \ x \geq 0 \\ 0, \ for \ x \leq 0 \end{cases} \tag{2.20}$$

Piecewise linear function

This function is represented as

$$f(x) = \begin{cases} 1, & if \ x \geq 0.5 \\ x, \ if & -0.5 > x > 0.5 \\ 0, & if \, x \leq 0 \end{cases} \tag{2.21}$$

where the amplification factor inside the linear region of operation is assumed to be unity. Out of these nonlinear functions, the sigmoid activation function is extensively used in ANN.

Multi layer perceptron

Multilayer perceptron (MLP) is a feed forward neural network, where the input signal propagates through the network in the forward direction on a layer by layer basis [18]. This network has been applied successfully to solve many non linear, complex and diverse problems in several fields. The structure of a three layer MLP (1-1-1) is shown in Fig.2.4. It consists of one input layer, one hidden layer and one output layer. The input to the network is represented by X_i. W_{ih} and W_{ho} represent

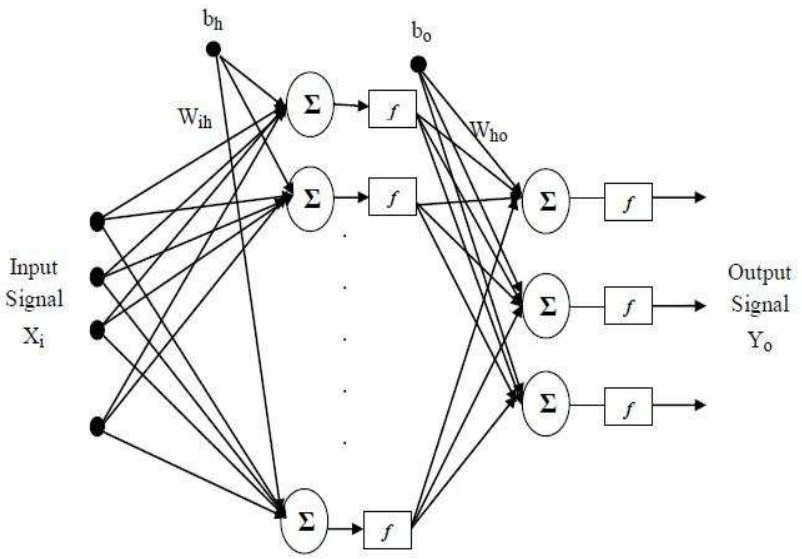


Figure 2.4: The structure of MLP network

the connecting weights between input layer to hidden layer and hidden layer to output layer respectively. The b_h and b_o represent the bias to neurons in hidden and output layers respectively. $f(.)$ represents the no-linear activation function for both hidden and output layers and N is the number of inputs at the input layer. The

output at each node of the hidden layer is computed as

$$a_h = f_h\left(\sum_{i=1}^{N} w_{ih}x_i + b_h\right) \tag{2.22}$$

Hence, the individual output of the neurons at the output layer is defined as

$$y_k = f_k\left(\sum_{h=1}^{n_1} w_{hk}a_h + b_k\right) \tag{2.23}$$

where the n_1 is the total number of neurons in the hidden layer. Thus the output of the MLP can be represented as

$$y_k = f_k\left(\sum_{h=1}^{n_1} w_{hk}f_h\left(\sum_{i=1}^{N} w_{ih}x_i + b_h\right) + b_k\right) \tag{2.24}$$

The weights and biases of different layers of MLP need to be learned in an efficient way to get the optimum of the objective function. Learning is an adaptive procedure by which the weights are systematically changed by a governing rule. Learning the networks can be of supervised , unsupervised and reinforcement type. Generally the learning algorithms may be classified into two categories: derivative based and derivative free. In this dissertation work the back propagation algorithm which is a derivative based algorithm is used for the learning of the neural networks. The back propagation algorithm is discussed in the subsequent sections.

Back propagation algorithm

Back propagation (BP) algorithm is the central to the supervised learning of MLP networks. The parameters of the neural network can be updated by BP in both sequential and batch mode of operation [19] [22]. In this algorithm, the weights and the biases are initialized as very small random values.The intermediate and the final outputs of the MLP are calculated by Eqs.(2.22)and (2.23) respectively. The output at the kth neuron of the output layer $y_k(n)$ is compared with the desired output $d_k(n)$, thereby the resulting error signal $e_k(n)$ is computed as

$$e_k(n) = d_k(n) - y_k(n) \tag{2.25}$$

The instantaneous value of the total error energy is computed by summing all errors squared over all neurons in the output layer as given by

$$\xi(n) = \frac{1}{2}\sum_{k=1}^{n_2} e_k^2(n) \tag{2.26}$$

where n_2 is the total number of neurons in the output layer.

The error signal produced by the comparison is used to update the weights between the layers and biases of the layers. The reflected error components at the hidden layer are determined by the errors of the last layer and the connecting weights between the hidden and last layer. These reflected error components are used to update the weights between the input and hidden layers and bias of the hidden layer. The weights and the biases are updated in an iterative method until the error becomes minimum.

The weights between input and hidden layer are updated according to the following equation

$$w_{ih}(n+1) = w_{ih}(n) + \Delta w_{ih}(n) \tag{2.27}$$

and update equation for weights between hidden and output layer is defined by

$$w_{hk}(n+1) = w_{hk}(n) + \Delta w_{hk}(n) \tag{2.28}$$

where $w_{ih}(n)$ and $w_{hk}(n)$ are the correction to the synoptic weights and are computed as

$$\begin{aligned} \Delta w_{hk}(n) &= -2\mu\frac{\partial\xi(n)}{\partial w_{hk}(n)} = 2\mu e(n)\frac{\partial y_k(n)}{\partial w_{hk}(n)} \\ &= 2\mu e(n) f_k^1\left(\sum_{h=1}^{n_1} w_{hk}a_h + b_k\right)a_h \end{aligned} \tag{2.29}$$

Similarly, the correction to other synaptic weight can also be computed. The biases are also updated in similar way as that of weights and the updated equations are given by

$$b_h(n) = b_h(n) + \Delta b_h(n) \tag{2.30}$$

$$b_k(n) = b_k(n) + \Delta b_k(n) \tag{2.31}$$

where $\Delta b_k(n)$ and $\Delta b_h(n)$ are the correction to biases of output and hidden layers respectively. The correction to bias of output layer $\Delta b_k(n)$ is calculated as

$$\begin{aligned} \Delta b_k(n) &= -2\mu \frac{\partial \xi(n)}{\partial b_k(n)} = 2\mu e(n) \frac{\partial y_k(n)}{\partial b_k(n)} \\ &= 2\mu e(n) f_k^1 \left(\sum_{h=1}^{n_1} w_{hk} a_h + b_k \right) a_h \end{aligned} \tag{2.32}$$

The correction to other bias which belongs to the hidden layer is also computed in the similar way.

2.3.2 Radial basis function network

Radial basis function network is a kind of nonlinear layered feed forward neural network in which the hidden units provide a set of functions that constitute an arbitrary basis for input patterns when they are expanded into hidden space [24]. The network is designed to perform a non linear mapping from the input space to the hidden space followed by a linear mapping from the hidden space to the output space [25]. The RBF networks are suitable for solving function approximation, system identification and pattern classification problems because of their simple topological structure and their ability to learn in an explicit manner [25] [26]. In the classical RBF network, there is an input layer, a hidden layer consisting of nonlinear node function, an output layer and a set of weights to connect the hidden layer and output layer. The input layer consists of the source nodes, which are also called sensory units that connect the network to its environment. The unique hidden layer in the network applies a nonlinear transformation from input space to hidden space using radial basis functions. The hidden space is of higher dimensionality in most of the applications. The response of the network supplied by the output layer is linear in nature. The basic architecture of the RBF network is shown in Fig. 2.5.

Here $x_i, \quad i = 1, 2, \cdots, M$ represents the input vector to the network, ϕ represents

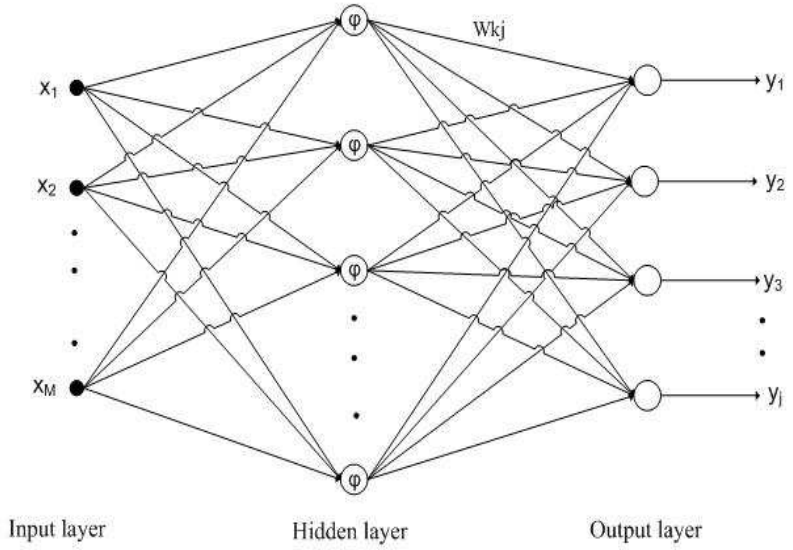


Figure 2.5: The architecture of radial basis function network

the radial basis function that perform the non-linear mapping and N represents the total number of hidden units. Each node has a centre vector c_k. W_{kj} represents the connecting weight between kth hidden unit and jth output unit and they perform linear regression. For an input feature vector $x(n)$, the output of the jth output node is given as

$$y_j = \sum_{k=1}^{N} W_{kj}\phi_k \tag{2.33}$$

Radial basis functions

The functional form of the radial basis functions $\phi(.)$, which is non singular is given by $\phi(x, c) = \phi(\|x - c\|)$, where $\|.\|$ denotes the euclidean norm. The radial basis functions generally used in the applications are described below.

Multiquadrics

$$\phi(r) = (r^2 + c^2)^{\frac{1}{2}} \qquad for \;\; c > 0 \;\; and \;\; r \in R \tag{2.34}$$

Inverse multiquadrics

$$\phi(r) = \frac{1}{(r^2 + c^2)^{\frac{1}{2}}} \qquad for \;\; c > 0 \;\; and \;\; r \in R \tag{2.35}$$

Gaussian function

$$\phi(r) = exp\left(-\frac{r^2}{2\sigma^2}\right) \qquad for \;\; \sigma > 0 \;\; and \;\; r \in R \tag{2.36}$$

In RBFNN, the three parameters that are to be updated are connecting weights between hidden and output units (W_{kj}), centre c_k and the Gaussian spread σ_k. These are updated by using the supervised learning method, which is similar to stochastic gradient algorithm. The cost function that is to be minimized is given by

$$\xi(n) = \frac{1}{2}\sum_{j=1}^{J} e_j^2(n) \tag{2.37}$$

where J is the total number of neurons in output layer, $e_j(n)$ represents the error signal which is the difference between desired output d_j and the output obtained y_j. Hence

$$\begin{aligned} e_j(n) &= d_j(n) - y_j(n) \\ &= d_j(n) - \sum_{k=1}^{N} w_k(n)\phi\left\{x(n), c_k(n)\right\} \end{aligned} \tag{2.38}$$

when the Gaussian function is chosen as the radial basis function, Eq. (2.38) becomes

$$e_j(n) = d_j(n) - \sum_{k=1}^{N} w_k(n) exp\left(-\frac{\|x(n) - c_k(n)\|^2}{\sigma_k^2(n)}\right) \tag{2.39}$$

According to stochastic gradient descent method [22], in order to minimize the cost function, the updated equations are as follows

$$w(n+1) = w(n) - \mu_w \frac{\partial}{\partial w}\xi(n) \tag{2.40}$$

$$c_k(n+1) = c_k(n) - \mu_c \frac{\partial}{\partial c_k}\xi(n) \tag{2.41}$$

$$\sigma_k(n+1) = \sigma_k(n) - \mu_\sigma \frac{\partial}{\partial \sigma_k} \xi(n) \tag{2.42}$$

Finally, the updated equations of the network is defined as

$$w(n+1) = w(n) + \mu_w e(n) \psi(n) \tag{2.43}$$

$$c_k(n+1) = c_k(n) + \mu_c \frac{e(n) w_k(n)}{\sigma_k^2(n)} \phi \left\{ x(n), c_k(n), \sigma_k \right\} \left[x(n) - c_k(n) \right] \tag{2.44}$$

$$\sigma_k(n+1) = \sigma_k(n) + \mu_\sigma \frac{e(n) w_k(n)}{\sigma_k^3(n)} \phi \left\{ x(n), c_k(n), \sigma_k \right\} \left\| x(n) - c_k(n) \right\|^2 \tag{2.45}$$

where $\psi(n) = \left[\phi \left\{ x(n), c_1, \sigma_1 \right\}, \phi \left\{ x(n), c_2, \sigma_2 \right\}, \cdots \phi \left\{ x(n), c_N, \sigma_N \right\} \right]$ is the hidden layer output and μ_w, μ_c, μ_σ are the learning parameters of the network.

2.4 Sensitivity and Specificity

Basically, to measure and compare the efficacy of a classifier, model or predictor, some statistical measures such as specificity, sensitivity, positive predictive value, negative predictive value and accuracy are evaluated through receiver operating characteristic curves (ROC). The ROC methodology is based on statistical decision theory and was developed in the context of electronic signal detection and problems with radar in the early 1950s. In recent years, it has been used in various areas like geophysics, electrical engineering, communication, medicine, biomedical, machine learning and data mining. ROC curves provide a global representation of the prediction accuracy.

In a predictor or binary classifier, for every instance of testing, there are four possible outcomes. If the instance is positive and it is classified as positive, it is counted as a true positive (TP). If the instance is positive and is classified as negative, it is counted as a false negative (FN). If the instance is negative and it is classified as negative, it is counted as a true negative (TN). If the instance is negative

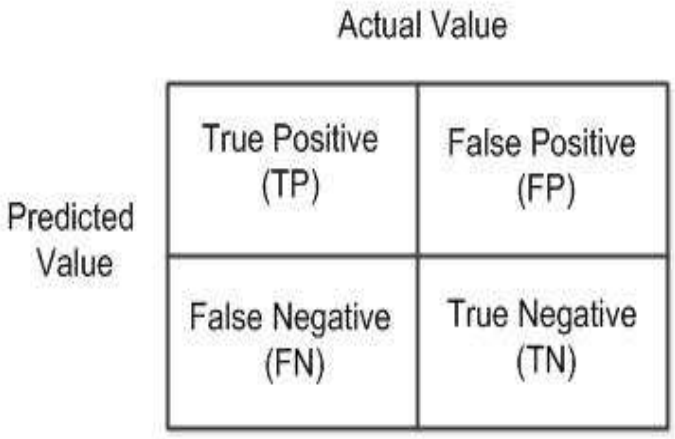


Figure 2.6: The process to evaluate sensitivity and specificity

and it is classified as positive, it is counted as false positive (FP). The statistical procedure to obtain all these terms is shown in Fig. 2.6.

Sensitivity

The sensitivity of the process is defined as the probability an individual be correctly classified when its real status is the one defined as positive, regarding the condition studied by the test.

Specificity

The specificity of the process is defined as the probability an individual be correctly classified when its real status is the one defined as negative. Hence,

Sensitivity (Sn) or True Positive Fraction (TPF)= TP/(TP+FN)

Sensitivity (Sp) = TN/FP+TN

False Positive Fraction (FPF)= FP/(FP+TN)

Positive predictive value (PPV) = TP/(TP+FP)

Negative predictive value (NPV) = TN/ (TN+FN)

The ROC curve relates the TPF as a function of FPF of a predictor or classifier

for varying threshold values. Basically the ROC curve plots for every possible decision threshold, which ranges from zero to the maximum value reached by the predictor, when computing the whole observations and the results are compared with the real values. The closer the ROC curve is to a diagonal, the less useful is the predictor. The more the curve moves to the upper left corner on the graph, the better the predictor. In this dissertation, we have used the ROC curves to evaluate the efficiency of the predictor developed to detect the patterns present in the genomic signals.

2.5 Conclusion

Of late it has been observed that detailed understanding and precise collection of information of bioinformatics require the knowledge of signal processing and soft computing tools. In the present research study, we have attempted to investigate on (*i*) protein coding region identification in DNA sequences (*ii*) hot spot identification in proteins (*iii*) protein structural class prediction and (*iv*) classification of microarray gene expression data. To facilitate in-depth investigation and accurate estimate, the techniques of DSP such as DFT, DCT, S-transform and soft computing techniques such as MLP, RBFNN have been employed. Therefore in this section these techniques have been briefly outlined so that understanding of the work carried out in the subsequent sections will be easier. Further these techniques have been judiciously selected so that they will be more useful in the respective application areas. The choice has been made based on the background which I had in the areas as well as from the literature available in these areas.

Chapter 3

Identification of Protein Coding Regions using Time-frequency Filtering Approach

Chapter 3

Identification of Protein Coding Regions using Time-frequency Filtering Approach

3.1 Introduction

A major goal of genomic research is to understand the nature of this information and its role in determining the particular function encoded by the gene. A key step in deciphering this goal is the identification of the gene locations and the protein coding regions in the DNA sequence. The enormous amount of genomic data that are available in public domain inspires the scientific community to process this information for the benefit of the mankind. The complete genomes provide essential information for understanding gene functions and evolution. The determination of patterns in DNA and protein sequences are also useful for many important biological problems such as identifying new genes, pathogenic islands and phylogenetic relationships among organisms. With the exponential growth of the genomic sequence, there has been an increasing demand to accurately identify the protein coding regions in the DNA sequence. The proliferation of computational methods in identifying the gene location in last two decades are quite encouraging and successful, but the efficiency of the prediction methods needs to be improved. Hence accurate prediction of genes has always been a challenging task for computational biologists, especially in eukaryote genomes [1] [3]. The 3-base

periodicity observed in the coding regions of various organisms has been used as a marker to identify the protein coding regions of genomes [27] [28]. In this chapter, we have proposed a novel time-frequency filtering approach to efficiently detect these protein coding regions in the DNA sequence.

3.1.1 Genes and Proteins

The DNA is a long double stranded molecule that encodes the structure of specific proteins in the cell, and also carries the information about how these proteins should be manufactured [1]. Functional elements or cellular instructions are coded within this string of characters. These instructions are recognized by cellular machinery and carried out during the growth and functioning of the cell. The DNA sequence is composed of four different nucleotides, namely adenine (A), cytosine (C), guanine (G) and thymine (T). The gene structure and expression mechanism in typical eukaryote cells are very complicated. The eukaryotic DNA is divided into genes and inter-genic spaces. Genes are further divided into exons and introns. The exons carry the code for the production of proteins, hence these are called as protein coding regions. The protein coding regions in DNA sequences are usually neither continous nor contigous. It is composed of alternating stretches of coding regions (exons) and non coding regions (introns). The structural relationship between DNA, exons and introns are shown in Fig. 3.1. When a particular instruction becomes active in a cell, the corresponding gene is turned on and the corresponding protein is produced through the transcription and translation process. This process of transcription and translation is common to all life and hence referred to as the central dogma of molecular biology [2]. The flow of genetic information to protein is shown in Fig. 3.2. During the transcription, the double stranded DNA is separated and an RNA template is generated by matching and chaining nucleotides complementary to that of DNA sequence. The introns are spliced out of the RNA chain to create a mature mRNA transcript. The mRNA nucleotides are then read in triplets (codons) and amino acids are produced by the universal genetic code through the translation

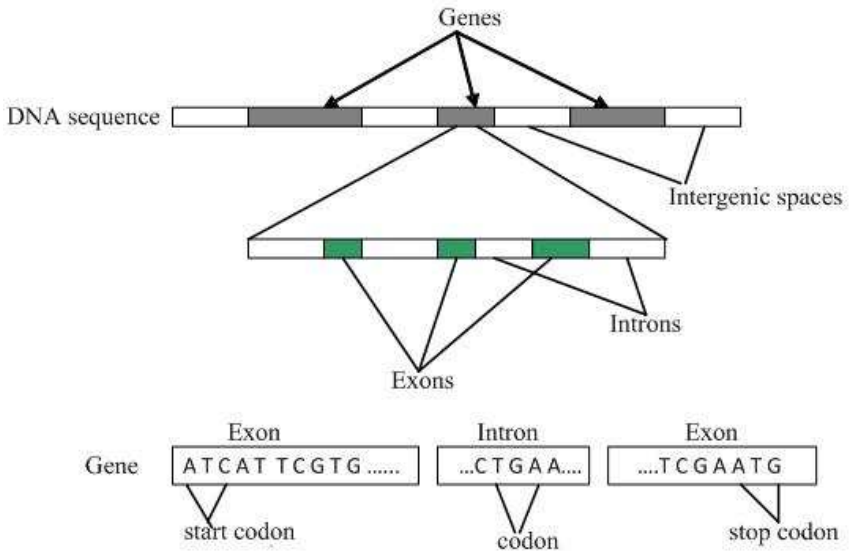


Figure 3.1: The relationship between the DNA sequence, gene, intergenic spaces, exons, introns and codons

mechanism which forms the protein. Therefore finding the coding regions in a DNA sequence involves searching of many nucleotides which constitute the DNA strand.

3.1.2 Fundamentals of 3-base periodicity in protein coding regions

The bases in the exon region can be divided into groups of three adjacent bases. Each triplet is a codon and hence a total of 64 codons are possible. Out of these 64 possible codons, only 20 amino acids are formed. The exons i.e. the coding regions within gene are denoted by start and stop codons. So by scanning the gene from left to right these codons of exons are spliced out to form a protein sequence. It has been found that the bases in the protein coding regions exhibit strong period-3 property due to the codon bias exist in these regions [28]- [31]. In a recent work, C. Yin and S. Yau [32] have elucidated that the three base periodicity (TBP) is not

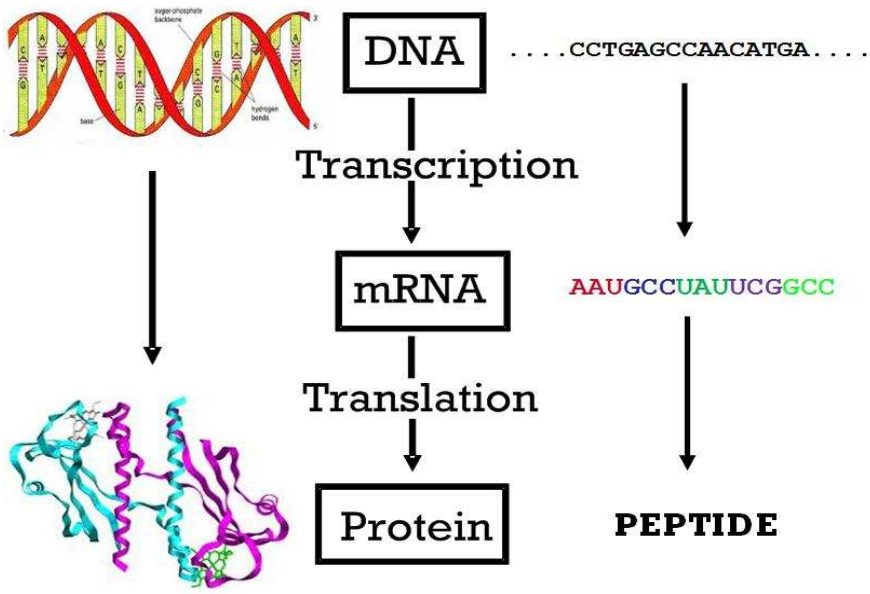


Figure 3.2: The central dogma of molecular biology (flow of genetic information from DNA to RNA to Protein)

determined by the genetic codon bias usage in DNA sequence. It is affected by the amino acid compositions but not by the ordering of the amino acids encoded by the DNA sequences. The codon frequencies or the unbalanced nucleotide distribution within the codons plays an important role to determine the three base periodicity in protein coding regions. Tiwari *et al.* [28] has observed that some genes do not exhibit period-3 behavior at all as in S. cerevisiae. Again in some Prokaryotes (cells without a nucleus), viral and mitochondrial base sequences such periodicity are also observed in non coding regions [1]. Due to these deviations and many other reasons, the identification of protein coding regions in the DNA sequence is a complex task. This periodic behavior relates to the short term correlation in the coding regions. In addition, there also exists a long-range correlation (so called 1/f spectrum) in the genome sequence which is considered as the background noise. The presence of this noise makes the task of gene finding problem even more complex. However the

3-base periodicity property has been used by many researchers as a good indicator to discriminate between the coding and non coding regions.

3.1.3 Review of the Gene prediction methods

Rapid and accurate determination of the exon locations is important for genome sequence analysis. Computational approach is the fastest way to find exons in the genomic DNA sequences. Many techniques have been proposed and proved successful in locating the exon regions present inside the gene in last two decades. Several model dependent methods like hidden markov model [33], neural network [34] [35] and pattern recognition [36] have been used to detect successfully the exons in the gene. These models are supervised methods which are based on some a prior information collected from the available databases. These methods are quite useful in the identification of coding region, but not always. There may be a chance that the sequenced organism may have coding regions that are not represented on the available databases. Also many model independent methods have been proposed to identify the coding regions in DNA sequences. Basically these studies are based on the Fourier spectral content [2,28,37], spectral characteristics [38] [50] and correlation of structure of DNA sequences [40]. Niranjan *et al.* [41] and Akhatar *et al.* [42] have proposed a parametric method of spectrum estimation based on autoregresive modeling. These methods require to define a prior analyzing window within which the spectrum of DNA sequence is to be computed. As a result it directly affects the efficiency and computational complexity of the predictor.

Hence there is a need for the development of alternative methods that can reduce the window length dependency and should be efficient. Recently Vaidyanathan *et al.* [43,44] have proposed to use digital filters to identify the coding regions. Also, Jamal *et al.* [45] have suggested a multirate DSP model for the same purpose. These model independent methods do not require the a prior window length and have shown to be effective in exon identification. But they could not attain satisfactory accuracy level. In order to solve this problem, a novel time-frequency filtering approach has

been proposed in this chapter. This method is independent of the window length constraint and employ a time-band filter to extract the period-3 component in the DNA sequence and thereby identify the coding regions in it. It is also robust to the background noise present in DNA sequence. Case studies on genes from different organisms have demonstrated that this method can be an effective approach for exon prediction.

3.2 Numerical mapping of DNA sequences

To apply suitable signal processing methods for the identification of protein coding regions, the character string of the DNA sequence is converted to a suitable numerical sequence. This is achieved by assigning a numeral to each nucleotide that forms the DNA sequence. Hence, different techniques have been suggested to achieve this particular conversion. The aim of each coding method is to enhance the hidden information for further analysis. One most widely used mapping is the Voss mapping [39], where the character string of DNA is converted to four binary indicator sequences for each base (A, T, C and G). It assigns a numeral '1' when a particular symbol is found in the sequence otherwise a '0'. Anastassiou [2] have proposed a complex number mapping by assigning a particular complex number to each base. Silverman et al. [46] have used a tetrahedron mapping in which each nucleotide is assigned to one of the four corners of a regular tetrahedron. Niranjan *et al.* [41] have proposed a real number mapping of the DNA sequence. Zhang *et al.* [47,48] presented a Z-curve mapping which is a three dimensional curve representation for the DNA sequence. Recently, an electron ion interaction potential (EIIP) indicator sequence [49] [51] have been used to map the character string of DNA to numeric form. The EIIP is defined as the average energy of delocalized electrons of the nucleotide. Assigning the EIIP values to the nucleotides, a numerical sequence is obtained which represents the distribution of the free electrons' energies along the DNA sequence. This has been successfully used to identify hot spots in proteins, for peptide design and also for identification of coding regions [49] [80]. The

EIIP sequence is a better choice for numerically representing DNA when compared to indicator sequences for the following reasons. First, it involves only a single sequence instead of four in the case of binary indicator sequences, thereby reducing the computational effort. Secondly, it is biologically more meaningful as it represents a physical property when compared to the indicator values, which represents just the presence or absence of a nucleotide. Hence, the EIIP representation method of numerical mapping of DNA sequence has been used in this work. The DNA sequence can be converted to the numerical sequence by replacing each nucleotide by the corresponding EIIP value. The EIIP values for the nucleotides are given in Table 3.1.

Table 3.1: The EIIP Values of the 4 nucleotides

Nucleotide	EIIP Value
A	0.1260
T	0.1335
G	0.0806
C	0.1340

For example, if $x[n] = AATGCATCA$, then using the values from Table 3.1 the corresponding numerical sequence is given as

x[n] = [0.1260 0.1260 0.1335 0.0806 0.1340 0.1260 0.1335 0.1340 0.1260]

3.3 Spectral content measure method

In this frequency domain method, the DFT of the EIIP indicator sequence is employed to exploit the 3-base periodicity [28] [2]. Let U[k] represents the DFT of the corresponding EIIP numerical sequence and is defined as

$$U[k] = \sum_{n=0}^{N-1} x[n] e^{-\frac{j2\pi nk}{N}} \tag{3.1}$$

for $k = 0, 1, \cdots, N-1$

Then the spectral content at kth instant is

$$S[k] = |U[k]|^2 \tag{3.2}$$

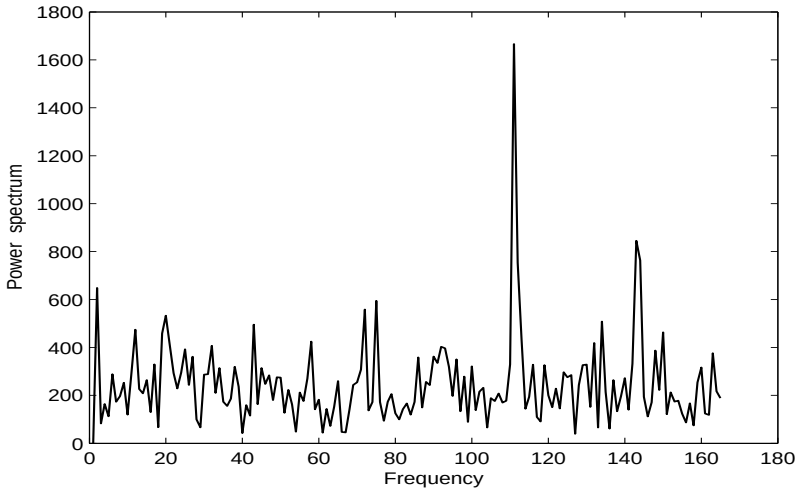


Figure 3.3: DFT power spectrum of the coding region of gene F56F11.4 (exon region from 2527-2857 of length 330 relative to the base position 7021). The peak at the frequency 330/3=110 corresponds to the period-3 component.

$S[k]$ acts as a preliminary indicator of a coding region indicating a peak at the $N/3$ frequency. To illustrate this behavior, the spectra of a coding and non-coding region of gene F56F11.4 of C. elegans chromosome III (a detail description is provided in section 3.6.1) is used. Figs. 3.3 and 3.4 show the spectra of the coding and no-coding regions of the gene. This procedure is used to detect the probable coding regions in the DNA sequence. Hence the coding regions are identified by evaluating $S[N/3]$ over a window of N samples, then sliding the window by one or more samples and recalculating $S[N/3]$. This process is carried out over the entire DNA sequence. The peaks in the spectra obtained by the sliding window DFT corresponds to the protein coding regions. We require that the window length (N) to be sufficiently large (typical sizes are a few hundred to a few thousand), so that the periodicity effect dominates the background noise spectrum. This approach increases the computational complexity as it computes the spectrum within a window and also

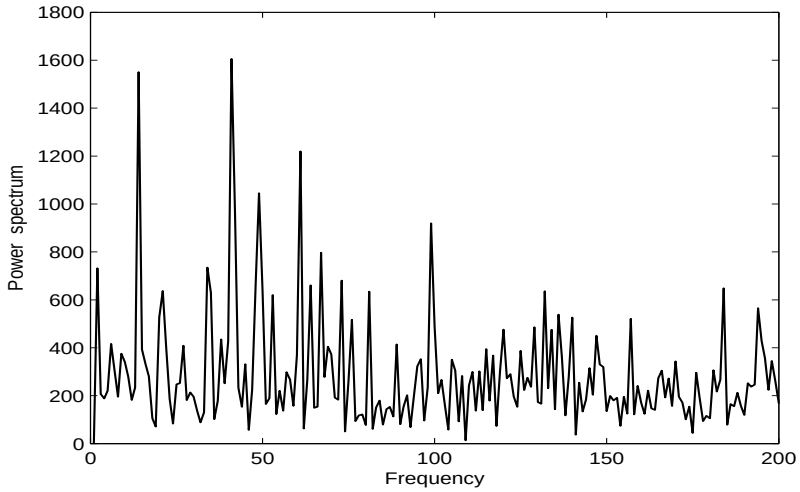


Figure 3.4: DFT power spectrum of non-coding region of gene F56F11.4 (intronic region from 1600-2000 relative to the base position 7021). No distinct peak is present in the spectrum.

constrained by the frequency resolution and spectral leakage effects of the windowed data record.

3.4 Digital filter method

The Fourier based spectral estimation method of protein coding region identification can be viewed as a digital filtering perspective [43]. The period-3 behavior of the coding regions is extracted by filtering the DNA sequence through a band pass filter $H(z)$ with pass band centered at frequency $2\pi/3$. The EIIP indicator sequence ($x(n)$) of the DNA sequence is passed through the filter $H(z)$ and the output sequence $y(n)$ is obtained. In the coding regions as it is expected to have period-3 component, a high energy particularly in these locations is produced. To enhance this feature, the power of the filtered sequence is computed as

$$Y(n) = [y(n)]^2 \tag{3.3}$$

Hence the plot of the $Y(n)$ against 'n' produces peaks in the coding regions and no peak in the intron regions. The design and implementation of $H(z)$ as an anti-notch

filter and its modifications are discussed in many papers [37] [44]. An overview of the implementation is presented here.

The IIR anti-notch filter

Consider a 2nd order all pass filter defined as

$$A(z) = \frac{R^2 - 2Rcos\theta z^{-1} + z{-}2}{1 - 2Rcos\theta z^{-1} + R^2 z^{-2}} \tag{3.4}$$

and a filter bank with two filters $G(z)$ and $H(z)$ obtained from the $A(z)$ defined as

$$\begin{bmatrix} G(z) \\ H(z) \end{bmatrix} = \frac{1}{2} \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix} \begin{bmatrix} 1 \\ A(z) \end{bmatrix} \tag{3.5}$$

Then $G(z)$ is written as

$$G(z) = k \left[\frac{1 - 2cosw_0 z^{-1} + z^{-2}}{1 - 2Rcos\theta z^{-1} + R^2 z^{-2}} \right] \tag{3.6}$$

where

$$cosw_0 = \frac{2Rcos\theta}{1 + R^2}, k = \frac{1 + R^2}{2} \tag{3.7}$$

When the radius R is less than and close to unity the $G(z)$ is a notch filter with a zero at frequency w_0. Also $H(z)$ and $G(z)$ are power complementary. Hence $H(z)$ can be a good anti-notch filter defined as

$$H(z) = \frac{1}{2} \left[\frac{(1 - R^2)(1 - z^{-2})}{1 - 2Rcos\theta z^{-1} + R^2 z^{-2}} \right] \tag{3.8}$$

The amplitude response of the antinotch filter with radius $R = 0.99$ is shown in Fig. 3.5. The DNA sequence can be viewed as a non stationary signal where the spectral components change along the sequence. Also it contains the background noise which comes due to the long range correlations among the bases on the DNA stretch. Under such situation the conventional Fourier domain filtering methods can not extract properly the occurrence of a period-3 component in the DNA sequence. Hence the joint time-frequency analysis is needed for analyzing such spectral content in the sequence.

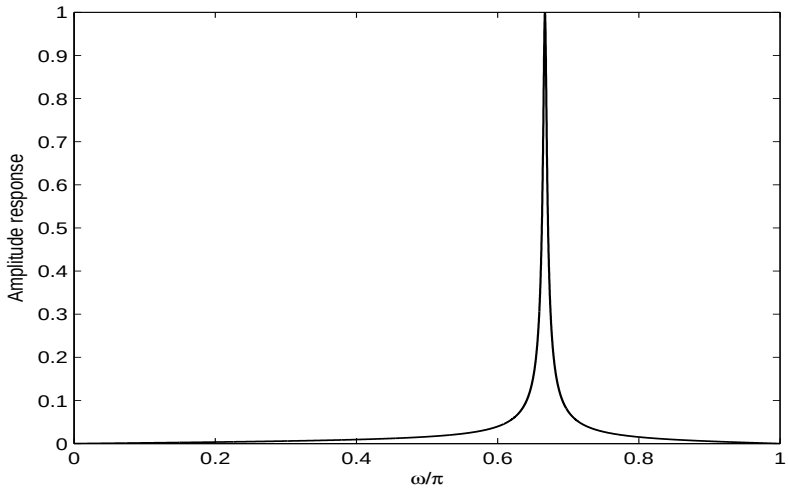


Figure 3.5: The anti-notch filter response

3.5 Statistical method of coding region identification

The statistical methods have found successful to detect the protein coding regions in DNA sequences. Fickett and Tung [27] have proposed various statistical determinants of protein-coding potential such as in-frame hexamer frequencies which were shown to possess good predictive power. Over the years, Markov models [33] and Bayesian pattern recognition algorithms have been found to be very efficient for gene modeling and used in several popular algorithms and programs such as HMMGene, Gene Mark, Gene ID, Genie etc. for prokaryotic as well as eukaryotic gene prediction.

Hidden Markov Model (HMM)

Hidden Markov models are general statistical modeling techniques for linear problems like sequences or time series and have been widely used in speech recognition applications. Following the success in speech recognition applications, it has been introduced for developing algorithms of pattern recognition in biological sequences such as protein structural modeling, sequence alignment etc. HMM is a finite model that describes a probability distribution over an infinite number of

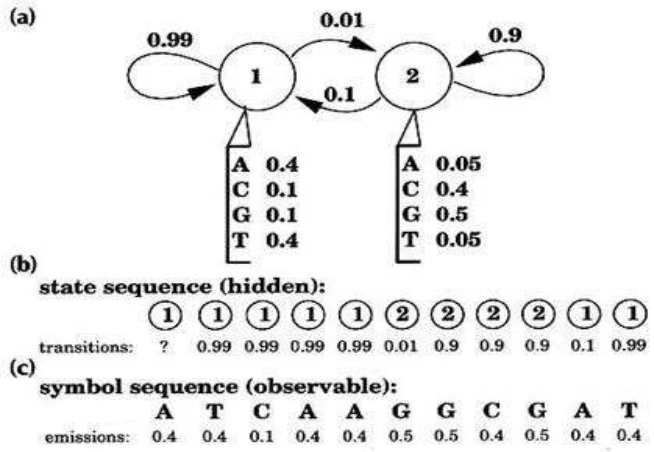


Figure 3.6: A simple hidden Markov model. A two-state HMM describing DNA sequence with a heterogeneous base composition is shown.(a) State 1 (top left) generates AT-rich sequence, and state 2 (top right) generates CG-rich sequence. State transitions and their associated probabilities are indicated by arrows and symbol emission probabilities for A, C, G and T for each state are indicated below the states.(b) This model generates a state sequence as a Markov chain and each state generates a symbol according to its own emission probability distribution (c). The probability of the sequence is the product of the state transitions and the symbol emissions.

possible sequences. It is composed of a number of states, which might correspond to positions in a 3D structure or columns of a multiple alignment. Each state 'emits' symbols (residues/nucleotides) according to symbol-emission probabilities and the states are interconnected by state-transition probabilities. Starting from some initial state, a sequence of states is generated by moving from state to state according to the state-transition probabilities until an end state is reached. Each state then emits symbols according to that state's emission probability distribution, creating an observable sequence of symbols. A simple hidden markov model for a DNA sequence is described in Fig. 3.6.

Basically an HMM introduces a state sequence $A = A_1, ..., A_n$, where A_i denotes the hidden states that 'emit' the observed (given) DNA sequence $S = S_1, ..., S_n$.

For example, the hidden states can be protein-coding, protein-coding shadow and non-coding in a hidden state model shown in Figure 3.6. As transitions between hidden states and emissions of nucleotides are governed by probabilistic rules, thus the state sequence(A) is most likely associated with the observed sequence(S). Mathematically, the state sequence could be found by maximization of the conditional probability P(A/S) with respect to A. This task is solved by a dynamic programming algorithm called the Viterbi algorithm or Bayes' rule.

3.6 The proposed time-frequency analysis method

Time-frequency analysis (TFA) is of great interest when the signal models are unavailable. In such cases, the time or the frequency domain descriptions of a signal alone cannot provide comprehensive information for feature extraction and classification [10]. Therefore, the time-frequency representation (TFR) has evolved as a powerful technique to visualize signals in both the time and frequency domains simultaneously. Several techniques have been proposed for this purpose as described in chapter 2. In this chapter, the S-transform has been proposed which possesses superior time-frequency resolution as well as frequency detection capability. A brief introduction to S-transform technique alongwith the superior time-frequency resolution capability is provided in chapter 2.

Due to the invertibility property of the S-transform, it can be suitably used for time-frequency filtering. The standard Fourier-domain filtering techniques are constrained to stationary pass bands and reject bands that are fixed for the entire duration of the signal. These methods may be adequate for the stationary signals where the signal component of the data and also the noise are time independent. However, many signals such as genomic signals are non-stationary in nature where the frequency response of the signal varies in time or time dependent noise exists.

To illustrate the limitation of conventional filtering methods let us consider a very low SNR (signal to noise ratio) synthetic signal. The time domain signal contains

two sinusoids: 6 Hz and 30 Hz. The 30 Hz signal is present during 1-30 and 65-128 samples and the 6 Hz signal present during 1-64 samples. A white random noise (n(t)) of strength -6 dB SNR is added to this signal. The time domain and frequency domain plots of the signal obtained by the use of DFT are shown in Fig. 3.7. The frequency domain plot indicates poor detection of 6 Hz frequency. Further, the 30 Hz signal which is present at two locations is observed only at one location in the spectral plot. In this type of application the conventional filtering fails to recover properly the desired signal.

Hence, for nonstationary signal there is a need for developing filters with time-varying pass bands and reject bands [16,17]. One of the most practical solutions to this problem is the joint time-frequency filter. In time-frequency filtering, the time frequency spectrum of a signal is first estimated and portions which are part of the noise are removed. Let the signal $x(t)$ is a sum of main signal component, $d(t)$ and noise component, $n(t)$ as

$$x(t) = d(t) + n(t) \tag{3.9}$$

Due to the linearity property of S-transform, it is written as

$$S(\tau, f) = D(\tau, f) + N(\tau, f) \tag{3.10}$$

where D and N are the S-transform of the main signal and the noise, respectively. Therefore, the filtering function $A(\tau, f)$ is to be such that

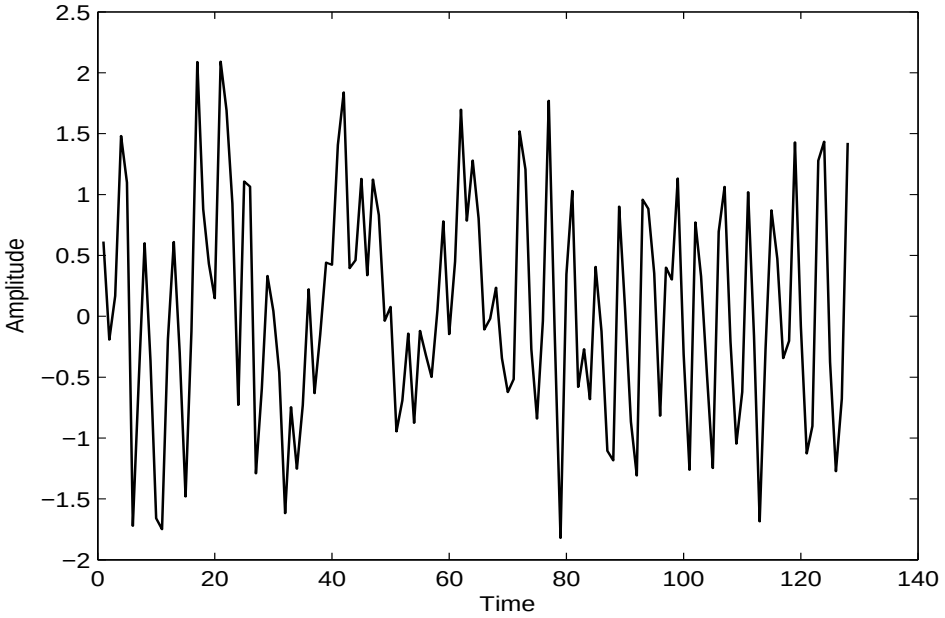
$$D(\tau, f) = A(\tau, f).S(\tau, f) \tag{3.11}$$

Using the inversion formula, the denoised signal $\tilde{x}(t)$ is recovered as

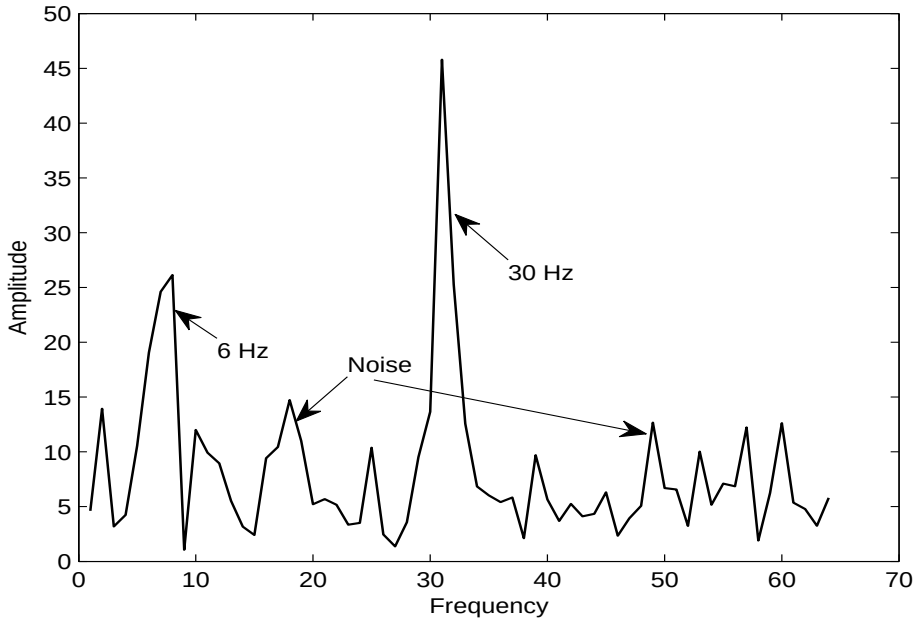
$$\begin{aligned} \tilde{x}(t) &= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} D(\tau, f)e^{j2\pi ft}d\tau df \\ &= \int_{-\infty}^{\infty} \tilde{X}(f)e^{j2\pi ft}df \end{aligned} \tag{3.12}$$

where $\tilde{X}(f) = \int_{-\infty}^{\infty} D(\tau, f)df$

Hence multiplying $S(\tau, f)$ with the filtering function $A(\tau, f)$ gives the S-transform of the denoised signal. The potential of the S-transform in localization and time-band



(a)



(b)

Figure 3.7: (a)A synthetic time series $h(t)$ is generated by $h = zeros(1, 128)$, $t_1 = 1 : 64, h(1 : 64) = cos\,(2\pi 6t_1/128)$, $t_2 = 65 : 128, h(65 : 128) = cos\,(2\pi 30t_2/128)$, $t_3 = 1 : 30, h(1 : 30) = h(1 : 30) + cos\,(2\pi 30t_3/128)$, $h(t) = h(t) + n(t)$ where n is the additive white noise of SNR -6 dB. (b)The amplitude spectra of $h(t)$

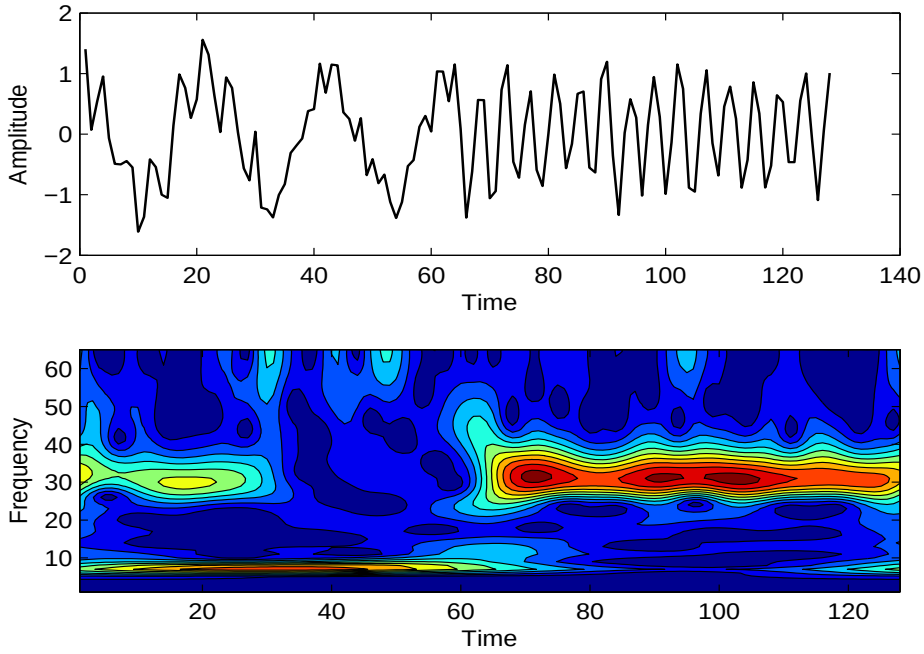


Figure 3.8: The synthetic time series and its S-transform spectrum

filtering is demonstrated by analyzing a synthetic time series which contains two sinusoids 6Hz and 30Hz at different locations and is contaminated by a noise of 0dB. The original time series and its spectrum obtained by S-transform are shown in Fig. 3.8. The localized signature of the two sinusoids is clearly elucidated from the spectrum. Now coming to the filtering point of view let us remove the frequency 30 Hz between samples 1 to 30. The three stages involved in the filtering using the S-transform are shown in Figs. 3.8-3.10. A boxcar window having a Hann tapering in the frequency direction [16] is used as the time-frequency filter. It has unit amplitude everywhere except the regions where the noisy signal present in the time-frequency plane. The time-frequency filter $A(\tau, f)$ is shown in Fig. 3.9.

It is clear from Fig. 3.10 that the 30 Hz signal present in the beginning which is considered as the noise, is completely removed from the time series and the S-transform spectrum of the recovered signal does not have that signal signature. It demonstrates that the S-transform itself does not include any artifact in the filtering

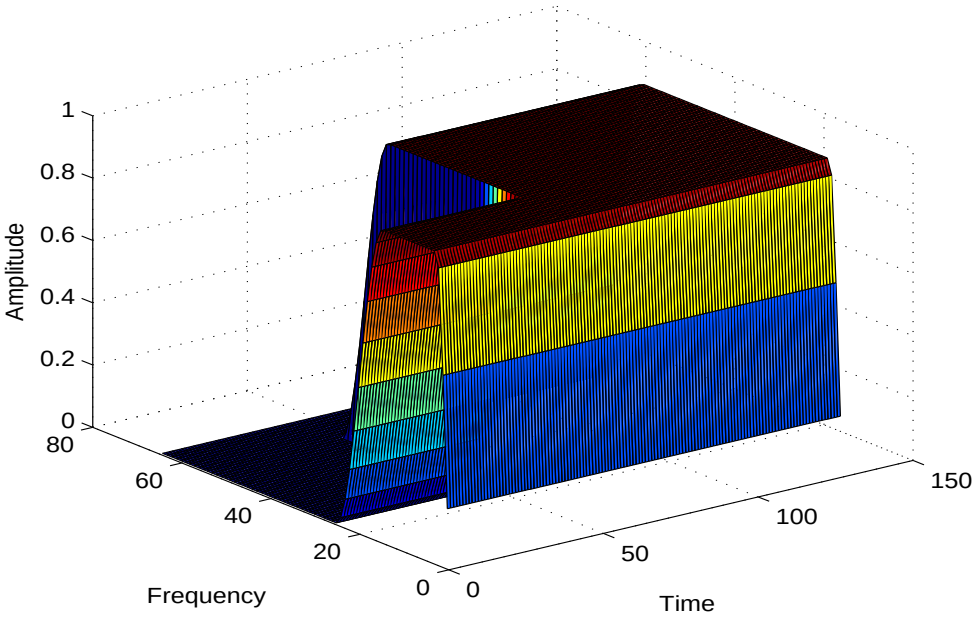


Figure 3.9: The time-band limited filter

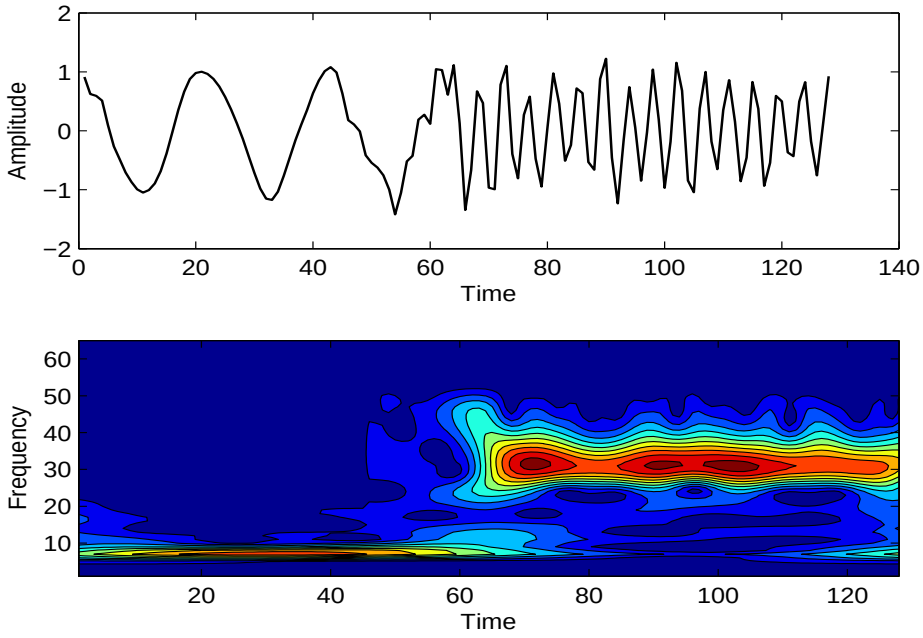


Figure 3.10: The recovered signal and its S-transform spectrum

process. In essence, the S-transform filtering can be effectively applied to identify the hot spots by picking up the characteristic frequency in the protein sequence.

In the present case, the period-3 signal in the genomic sequence is considered as the signal of interest and the rest is treated as noise. Hence, the time-frequency filtering technique is used as a potential candidate to extract the protein coding regions in the DNA segment.

3.6.1 Identification of protein coding regions in DNA using S-transform based filtering approach

The nucleotides are assigned by the corresponding EIIP value as given in Table 3.1, which provides a numerical sequence of the DNA. Then the spectrum of the DNA sequence under consideration is computed to observe the distribution of the energy of the frequency components throughout the sequence. A view of the spectral distribution obtained by the proposed method for gene F56F11.4 of C. elegans chromosome III is shown in Fig. 3.11. In the S-transform spectrum the high energy regions (bright areas) in the DNA sequence correspond to the dominant frequencies in the DNA sequence. As the period-3 frequency is dominant in coding regions, it provides distinct energy concentrated areas in the time-frequency plane where that frequency is present which are marked by rectanglear boxes in Fig. 3.11. Then a specific band limited time-frequency filter (mask) is designed to separate the frequency of interest. The whole process of S-transform based filtering approach for protein coding region identification is depicted in a flow graph in Fig. 3.12. The complete step-by-step procedure of the proposed S-transform based filtering for identification of hot spots is outlined in sequel:

1. Convert the DNA sequence of interest into a numerical sequence using the EIIP values (Table 3.1).

2. Compute the spectrum of the DNA sequence using the S-transform technique.

3. Design the band limited filter (mask) in time-frequency domain which

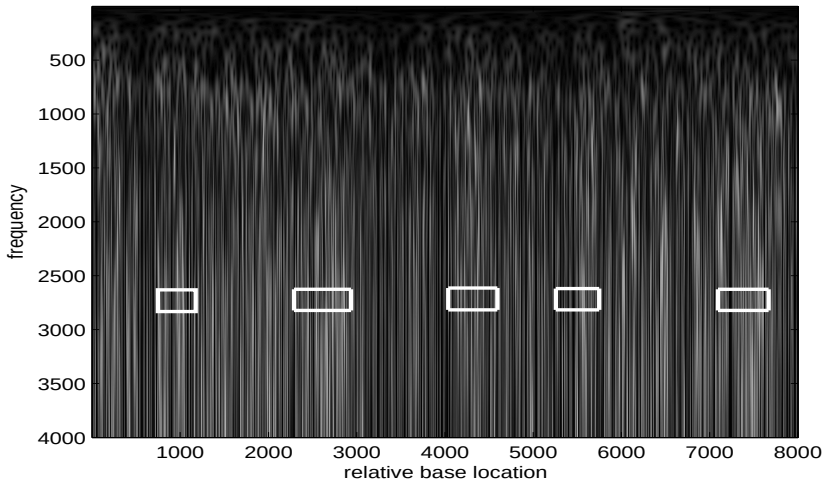


Figure 3.11: Spectrogram of the DNA sequence of F56F11.4. The high spectrum values (bright regions) correspond to the dominant frequency components. The coding regions which are relevant to the period-3 frequency are indicated by rectangular boxes in the spectrum.

selects the period-3 frequency and activate during the specific regions in the time-frequency plane.

4. Filter the DNA numerical sequence of interest by using the time-frequency filter.

The peaks in the energy of the filtered output signal identify the locations of the protein coding regions. If the output signal is denoted as $y(n)$, then its energy is given as

$$E(n) = |y(n)|^2 \tag{3.13}$$

This energy is referred to as the energy sequence corresponding to three base periodicity of the DNA sequence. Then the coding regions are predicted by thresholding the energy sequence.

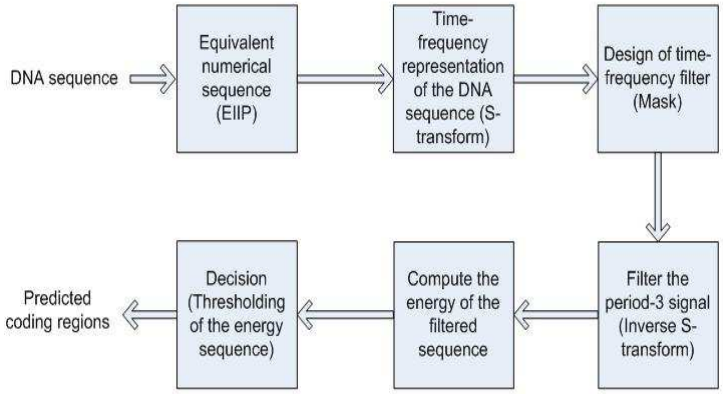


Figure 3.12: The flow graph of the S-transform based filtering approach for protein coding region identification

3.7 Results and Performance evaluation

3.7.1 Data resorces

In this work, analysis of eukaryotic DNA sequences has been studied in the context of coding region identification. For demonstration purpose the DNA sequence of gene F56F11.4 of Caenorhabditis elegans chromosome III [Gene bank: AF099922] has been used. C. elegans is a free living nematode (roundworm), about 1 mm in length, which lives in temperate soil environment. This has been used as a benchmark problem for different gene detection techniques and known to have five distinct exons, relative to nucleotide position 7021 according to the national center for Biotechnology information (NCBI) data base. The relative positions of the coding regions are 928-1039, 2528-2857, 4114-4377, 5465-5644 and 7265-7605. For the detailed analysis we have also studied the HMR195 benchmark dataset which consists of 195 single gene (either single or multi exon) sequences of human, mouse and rat [52]. The characteristic of the dataset is described as follows:

1. The ratio of human:mouse:rat sequences is 103:82:10.

2. The mean length of the sequences in the set is 7,096 bp.
3. The number of single-exon genes is 43 and the number of multi-exon genes is 152.
4. The average number of exons per gene is 4.86.
5. The mean exon length is 208 bp, the mean intron length is 678 bp.
6. The proportion of the coding sequence in this dataset is 14%, the intronic sequence is 46%, and the intergenic DNA is 40%.

The HMR195 dataset is available at http://www.cs.ubc.ca/labs/beta/genefinding/.

3.7.2 Experimetal result

To demonstrate the performance of the proposed method the DNA sequence of the gene F56F11.4 of C. elegans chromosome III is analyzed. In this chapter, the existing model independent methods such as conventional sliding window DFT, the IIR anti-notch filter and statistical method such as hidden markov model are also simulated and the results obtained are compared with those obtained by the proposed method. The simulation results of gene F56F11.4 of the model independent methods are presented in Fig.3.13 for comparison purpose.A graphical representation of the EIIP coded sequence of gene F56F11.4 is presented in annexure-I. In the DFT spectrum analysis, a rectangular window of length 351 bp and step size of 1 is used. The peaks in the spectrum correspond to regions where three base periodicity is present. Hence, the coding regions are identified by putting a threshold to the spectrum or filtered energy sequence. The regions having energy above the threshold are considered as the protein coding regions. As the non coding regions do not have a period-3 property, the energy in that region is low which is demonstrated in the Fig. 3.13. It is interesting to note that the first coding region of 112 bp along the positions 929-1039 has a weak TBP and the remaining four coding regions present high TBP. The spectral content method and anti-notch filter fail to detect properly

that region, but the S-transform filtering approach catches up that region better than these two methods. In order to have a comparison of the efficiency of these methods the threshold percentiles from 1 to 99 are used on the measures of the individual methods for the identification of probable coding regions. Hence, the statistical parameters such as sensitivity, specificity and average accuracy [42] are calculated under the same conditions at different threshold values. These measures have also evaluated for HMM method. The best result achieved in each method with the corresponding threshold value is listed in Table 3.2. The proposed method provides the best performance at a threshold of 85 percent with a sensitivity of 0.88, specificity of 0.98 and average accuracy of 0.96. A comparative analysis of the average accuracy against the threshold values for the three model independent methods is shown in Fig. 3.14. Further a comparative study of the exon locations obtained from these four methods with that reported in NCBI database is listed in Table 3.3. It shows that the proposed method provides better discrimination between the exons and the introns compared to those offered by the DFT, anti-notch filtering and HMM methods. To assess the performance of the three model independent methods, the receiver operating characteristic (ROC) curves are also obtained. It is a representation of the prediction accuracy of separation of exons and introns in the gene. The ROC curve relates the true positive rate as a function of false positive rate for varying threshold values. The ROC curves for all the three methods are shown in Fig. 3.15. The closer is the ROC curve to a diagonal, the less effective is the method for discriminating between exon and intron. More steep the curve towards the vertical axis and then across, the better is the method. A more precise way of evaluating the performance is to calculate the area under the ROC curve (AUC). The closer is the area to 0.5, the less effective is the method and closer to 1.0, the better is the method. The area under the ROC curve for the S-transform filtering method is found to be 0.9288 and the same for the DFT and anti-notch filter methods are 0.8615 and 0.8369 respectively. Hence the proposed S-transform filtering method of exon prediction outperforms other methods as it offers highest

Table 3.2: Position comparison study of the exons of F56F11.4 by the DFT, anti-notch, S-transform filter and HMM methods.The length of the exons are shown in the braces.

Position in Genebank(NCBI)	DFT based approach	Anti-notch filtering	S-transform filtering	HMM method
929-1039(110)	936-1169(233)	942-1164(222)	974-1037(63)	968-1092(125)
2528-2857(330)	2573-3005(432)	2538-2956(418)	2539-2908(369)	2566-2906(341)
4114-4377(264)	4073-4432(359)	4132-4462(330)	4076-4409(333)	4088-4393(306)
5465-5644(180)	5467-5658(191)	5497-5672(175)	5454-5644(190)	5483-5667(185)
7255-7605(351)	7396-7806(410)	7406-7728(322)	7305-7597(292)	7348-7692(345)

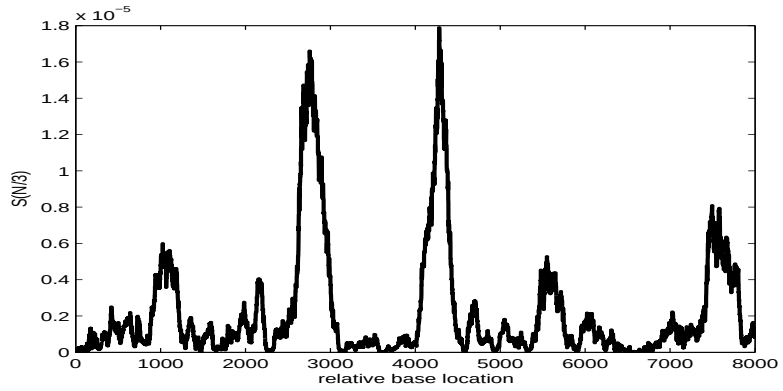
Table 3.3: Summary of the best performance (accuracy) of identification of coding regions in F56F11.4 using the DFT, anti-notch,S-transform filter and HMM methods.

Method	Sensitivity	Specificity	Average accuracy	Threshold
S-transform filter	0.88	0.98	0.96	85
DFT based approach	0.82	0.86	0.85	81
Anti-notch filter	0.81	0.82	0.82	82
HMM	0.82	0.84	0.83	—

area under the curve.

Further, several DNA sequences from the benchmark dataset HMR195 have been studied. The gene AF009614 has taken for demonstration and the power spectrum obtained from all the three methods are shown in Fig. 3.16. The gene AF009614 has two exon regions at positions 1267-1639 and 3888-4513 in the sequence. From this figure, it is clearly elucidated that the proposed S-transform based filtering method offers improved performance compared to its counterparts. A classification experiment has also been carried out to compare the efficiency of the proposed method. From the HMR195 dataset, 50 sequences whose average exon length is greater than 200 bases are chosen for the experiment.

Total 222 coding sequences and 237 non coding sequences are used in the



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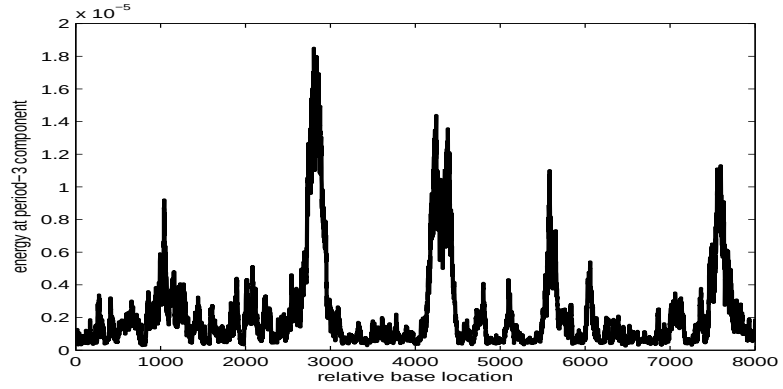

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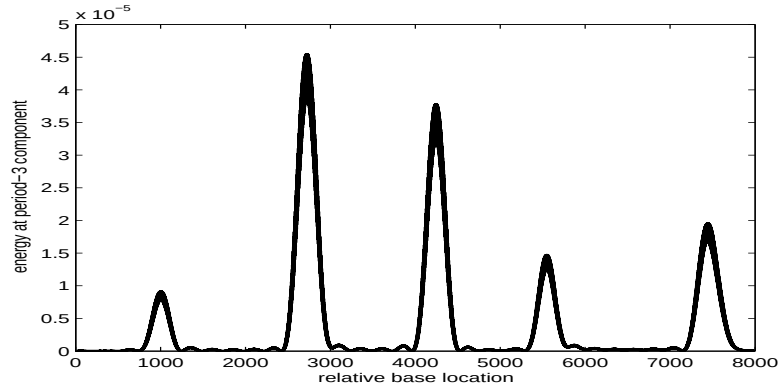


(c)

Figure 3.13: Comparison of the power spectra of gene F56F11.4 obtained by DFT, anti-notch filter and S-transform filter. (a)Spectral plot of gene F56F11.4 using DFT (b)Spectral plot of gene F56F11.4 using anti-notch filter (c)Spectral plot of gene F56F11.4 using S-transform filter.

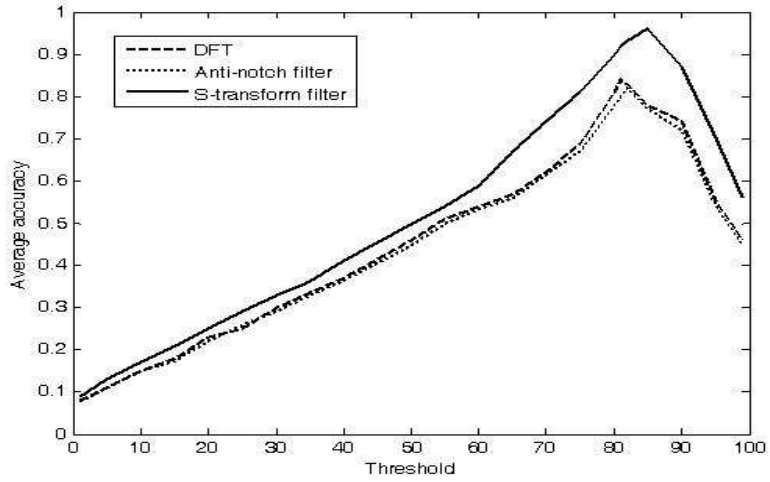


Figure 3.14: Average accuracy of identification versus threshold of the gene F56F11.4

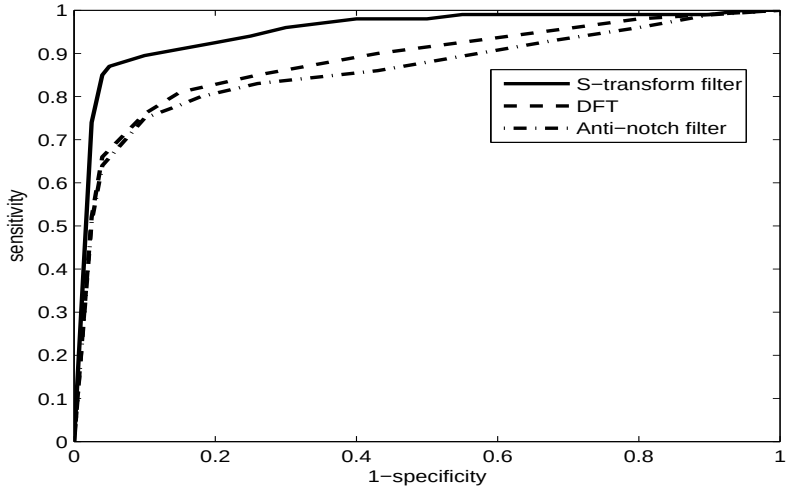
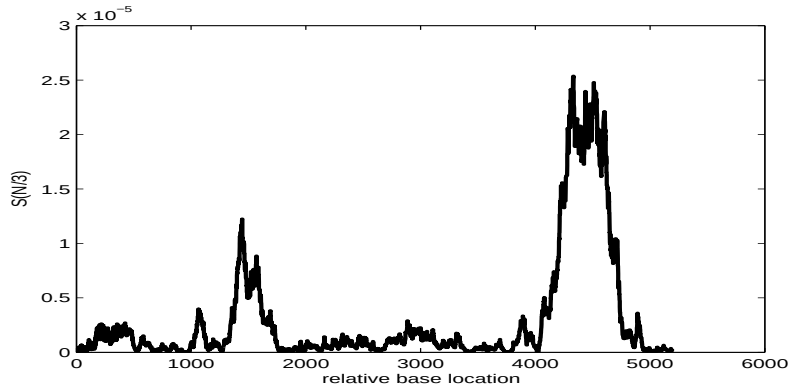


Figure 3.15: ROC curves obtained by DFT, anti-notch filter and S-transform filter of the gene F56F11.4

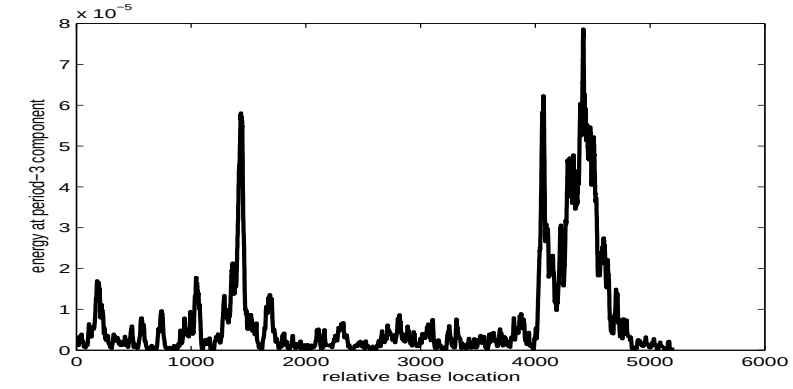
study.The threshold percentile of 1- 99 is used to discriminate the coding regions from the non coding regions. Accordingly the ROC curves by the three methods are obtained as shown in Fig. 3.17. The areas under the ROC curve are also calculated. These are 0.8602, 0.8316 and 0.8094 for the proposed, DFT and anti-notch filter methods respectively. Hence the S-transform based filtering method presents a better performance on the classification, thereby the superiority of the proposed method is assessed.

3.7.3 Discussion

The existing exon identification methods employ a variety of biological information and coding techniques in association with many computational methods to predict the exon regions in DNA. Still the 3-base periodicity pattern has been used as a basis to identify the coding regions. In this chapter a new time-frequency filtering scheme based on the TBP has been proposed for the identification of protein coding regions. The S-transform method provides a pictorial view of the energy distribution of the frequencies with time which helps in the analysis of the spectral varying signal. It gives a multi resolution view of the signal so that distinct patches of periodic signal can be analyzed easily.It is a model independent method which does not require any training sample to predict and also independent of the window length constraint for proper computation of the spectra of coding regions. The multi resolution analysis of the signal enables the proposed method to be effective for both small and larger coding regions. Another aspect of this study is that the EIIP can be used as an efficient coding scheme for DNA sequence analysis. The proposed method is found to be robust against the background noise which occurs due to long range correlation of bases in the DNA sequence. Thus the coding regions are better discriminated from the non coding regions and thereby the accuracy of identification increases considerably. Although the proposed method achieves better accuracy in the identification of the coding regions, it requires more computational effort. Another limitation of the S-transform method is that it provides low frequency resolution



(a)



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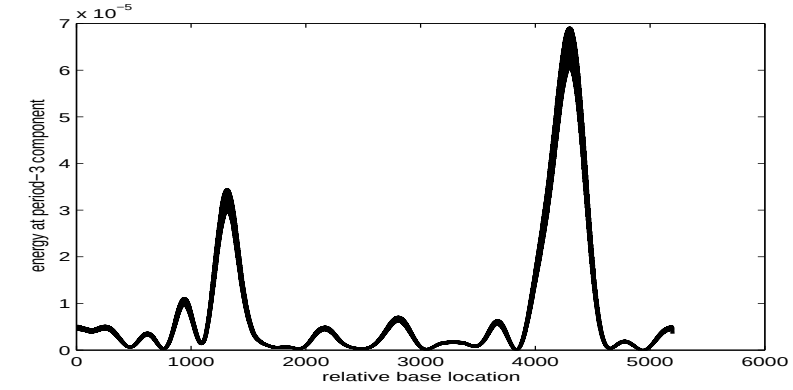

(c)

Figure 3.16: Comparison of the power spectra obtained by the three methods for gene AF0099614. (a) Spectral plot of gene AF0099614 using DFT (b) Spectral plot of gene AF0099614 using anti-notch filter (c) Spectral plot of gene AF0099614 using S-transform filter.

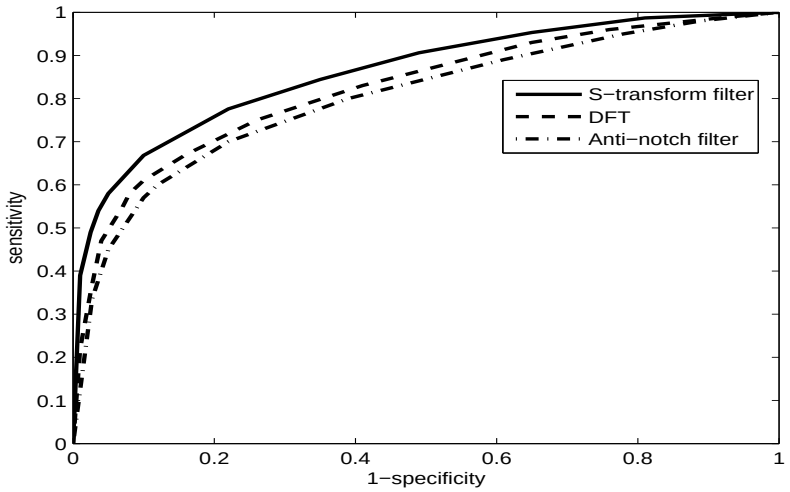

Figure 3.17: ROC curves obtained by the DFT, anti-notch filter and S-transform filter (From 50 sequences of HRM195 dataset)

at higher frequencies and low time resolution at lower frequencies. This occurs due to the scaling nature of the Gaussian window during spectrum computation, which may affect the time-frequency filtering operation and also the accuracy. Hence, improvement of the resolution of the spectrum can further improve the prediction accuracy.

3.8 Conclusion

Due to lack of the exact knowledge about the sequence features between coding and non-coding regions, the identification of protein coding regions in DNA sequence has been a challenging issue in bioinformatics. In this chapter, an efficient time-frequency filtering approach is proposed for the identification of coding regions in the DNA sequence. The proposed method employs a multi resolution approach to analyze both the small and large coding regions and it does not depend on a prior window length as in case of Fourier methods. The performance of the proposed method is compared with the existing methods and the results show its superiority in identification of the exon regions.

Chapter 4

Localization of Hot Spots in Proteins using a Novel S-transform based Filtering Approach

Chapter 4

Localization of Hot Spots in Proteins using a Novel S-transform based Filtering Approach

4.1 Introduction

Biological mechanisms of living organisms like metabolism, gene regulatory and interaction networks have put numerous challenges to modern biomolecular research. In particular, identification and characterization of protein-protein interactions is a burning issue in protein science. Proteins are the basic building blocks of all living organisms and protein-protein interactions are the basis of all biological processes, both inside and outside the cell [53] [54]. The protein is made up of amino acids. There are twenty amino acids and are represented in a protein sequence as a string of alphabetical symbols with typical lengths ranging from 100 to 10000 [2]. The protein molecules fold beautifully to form a highly specific 3-dimensional shape, which defines their particular biological activities. The 3-D structure of a protein is important because the structure is linked with the biological function. This 3-D shape allows the protein to interact with other molecules known as targets at specific sites which are referred to as active sites of protein [55] [56]. In active sites of protein, there are certain residues (amino acids) that operate as an interface in the binding and recognition between interacting molecules [58] and are termed as hot spots. Basically the target molecules are proteins, DNA stretches or some

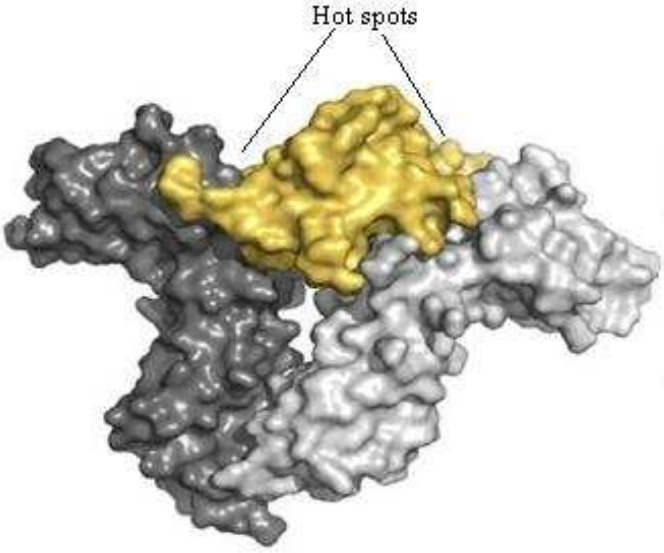

Figure 4.1: A schematic view of the hot spots in the complex of human growth hormone and its receptor.The human growth hormone (yellow) bound to the extracellular portion of its homodimeric receptors (grey). Available online at: doi:10.1371/journal.pcbi.0030119.g001

other small molecules. The search for protein functions provides the identification and characterization of each protein as well as in-depth knowledge regarding their interaction with other proteins and DNA molecules. The protein-protein interaction or hot spot identification provides a base to identify and analyze the drugs, molecular medicines, etc. The identification of protein hot spots and solving the protein structure-function problem [57] is a challenging task for researchers in biology, engineering and computer science. Many protein-interaction networks have been modeled to discover the mechanism of protein complexes, but a deep understanding of this requires the knowledge of interface amino acids that takes action in protein-protein interactions [59]- [61]. A biological experimental technique known as Alanine Scanning Mutagenesis (ASM) has been used to identify the hot

spots [62]- [64]. It uses the measure of the energy contribution of interface amino acids by mutating each amino acid to alanine. There is a little bit ambiguity because a single mutation cannot infer the effort in interaction as the protein structure and its interactions are highly complex to be summed as the features of individual residues. However, the alanine scanning is considered as a good method of identification of hot spots and also it is widely accepted by many researchers. The alanine is chosen because it eliminates its side chain easily without altering the main chain conformation as the side chain does not directly involve in protein function. It also does not put any extreme electrostatic or steric effects on the main chain conformation.

The protein-target interaction is very specific in nature. The protein binds to the target in an analogous manner as a key fits to the corresponding lock. A schematic view of the interaction of a protein with target through the hot spots is shown in Fig.4.1. As the interaction involves binding of the protein to the target it releases some energy in that process known as binding free energy (ΔG). When the interface amino acid is mutated to the alanine, the binding free energy of the mutated protein-target complex is measured. Then the change in the binding energy ($\Delta\Delta G$) before and after the mutation is evaluated. It has been demonstrated that if $\Delta\Delta G$ is more than a threshold (2.0 kcal/mol) by the mutation of an amino acid, then it is considered as a hot spot [62] [65]. This concept has also been accepted by the biologist and used by the researchers. The ASM procedure is very expensive as it involves wet lab experiment which needs variety of chemicals, instruments etc. It is also time consuming and requires a lot of effort. Hence there is a need of advanced computational techniques to make this task easier in identifying the hot spot locations [58] [77]. The outcome of the computational techniques provides a step to combat the localization problem and avoids the unnecessary mutations in wet lab experiments.

4.2 Review of hot spot identification methods

Several structure and sequence based computational methods have been proposed in the literature to identify the hot spots in proteins. Kortemme and Baker [66] [67] proposed a computational alanine scanning method known as Robetta-Ala, which use a physical model to calculate the energy of the interaction of the mutation to alanine. Many feature based classification models have been proposed to predict the hot spots. Ofran and Rost [68] [69] proposed a feature based method, called ISIS, which predicts the hot spots from the protein primary sequence only. It uses the physicochemical features, evolutionary and structural features of the protein through neural network model to predict the hot spots. Recently Darnell *et al.* [70] [71] proposed a model which uses a physical and knowledge based approach to predict the binding hot spots. Guney *et al.* [72] predicts the hot spots using the residue conservation and solvent accessible surface area (ASA). Burgoyne and Jackson [73] used the van der Waals potential, electrostatic potential, desolvation and surface conservation properties of residues to define hot spots on the protein surface. Tuncbag *et al.* [74] used the conservation, solvent accessibility (ASA) and statistical pairwise residue potential of interface residues to predict the hot spots in proteins. Ma *et al.* [75] and Keskin *et al.* [76] identified the hot spots by analysing the structurally conserved residues in protein. Chao et al. [59] have applied support vector machine (SVM) to predict hot spots using sequence, structure and molecular interaction information based features. Xia et al. [77] also employed SVM with protrusion index and solvent accessibility feature of the protein to identify the hot spots. Although these methods are useful for the prediction, they need a large number of samples for training the model and the accuracy may decrease when applied to a large set of protein complexes.

However, the hot spots in protein can also be identified by the use of resonant recognition model (RRM) which correlates the biological functioning of the protein to the characteristic frequencies [see section 4.3]. These hot spots in protein can be localized where the characteristic frequencies of the functional groups are dominant.

Each such frequency in the spectral domain signifies a protein function. The signal processing techniques are the suitable candidates to extract these characteristic frequencies in the protein sequence, which are based on the sequence information only. Recently, a signal processing technique known as digital filtering [82] [83] has been applied for this purpose. A protein sequence is usually noisy and may contain the hot spots (characteristic frequency) at different locations along the protein sequence. Under such situation, if the conventional digital filtering technique is applied, it fails to detect uniquely all characteristic frequencies present in the protein sequence. Secondly, if the same characteristic frequency is present in more than one location, the conventional approach of spectral detection identifies the frequencies, but not the locations at which they occur.

The real life protein sequences are usually characterized by noisy signals and hence, the use of existing filtering methods to such sequences do not provide accurate localization and identification of characteristic frequencies. In these applications, the time-frequency analysis and filtering are required to achieve accurate and effective solution. In the DSP literature many time-frequency analysis methods, such as STFT [11], WT [12] and S-transform [17] have been proposed which localize the events in the signal. A brief introduction to these techniques is provided in chapter 2. Among these, the S-transform possesses superior time-frequency resolution as well as frequency detection capability. Therefore, the motivation of the present work is to propose S-transform to detect efficiently all the characteristic frequencies present in protein and to identify the corresponding hot spot locations.

4.3 Resonant recognition model

Biological functions of proteins are primarily determined by a model of protein-target interactions known as resonant recognition model (RRM). The RRM is a physico-mathemati-cal approach that interprets protein sequence information using signal analysis methods. According to this model, there is a significant correlation between the spectra of the numerical presentation of amino acid sequences and their

biological activities [78] [79]. To apply suitable signal processing methods for the analysis, the character string of protein need to be converted to a suitable numerical sequence. This is achieved by assigning a numeral to each amino acid that forms the protein.

The assignment of numerical value to each amino acid is based on some physical properties that are relevant to the protein's biological functioning. A variety of amino acid indices have been reported in literature. Cosic *et al.* [80] have demonstrated that the best correlation can be obtained with the parameters, that are related to the energy of delocalized electrons of each amino acid which have strongest impact on the electronic distribution of the whole protein. An effective way of assigning the numerical value is the electron-ion-interaction potential (EIIP). The EIIP is defined as the average energy of delocalized electrons of the amino acid which can be evaluated by the pseudo potential model reported in [80]. The EIIP values for the 20 amino acids are listed in Table 4.1. Hence the primary sequence of protein can be converted to the numerical sequence by replacing each amino acid by the corresponding EIIP values. Veljcovic *et al.* [81] have reported that the

Table 4.1: EIIP values of the 20 amino acids

Amino acid	EIIP	Amino acid	EIIP
Leucine (Leu)	0.0000	Trypsin(Try)	0.0516
Isoleucine(Ile)	0.0000	Tryptophan(Trp)	0.0548
Asparagine(Asn)	0.0036	Glutamine(Gln)	0.0761
Glycine(Gly)	0.0050	Methionine(Met)	0.0823
Valine(Val)	0.0057	Serine(Ser)	0.0829
Glutamic acid(Glu)	0.0058	Cystrine(Cys)	0.0829
Proline(Pro)	0.0198	Threonine(Thr)	0.0941
Histidine(His)	0.0242	Phenylalanine(Phe)	0.0946
Lysine(Lys)	0.0371	Arginine(Arg)	0.0959
Alanine(Ala)	0.0373	Asparatic acid(Asp)	0.1263

Fourier spectral analysis of the EIIP sequence of the protein has strong relevance to its functional activity. All the proteins belonging to a functional family share

a common spectral component, which characterizes a particular function of the group. This component is defined as the characteristic frequency of the functional group. In protein-target interaction both the protein and target share the same characteristic frequency, but are opposite in phase. This is believed to provide a resonant recognition in the binding process and hence the mechanism termed as RRM. The analysis of the function of protein using RRM is generally performed in two stages. First the symbolic sequence of the protein is converted into the numerical sequence using the EIIP values. Then the discrete Fourier transform of the proteins is computed to evaluate the consensus spectrum. It has been observed that the spectra of a family of protein sequences sharing a common frequency show a peak in the cross-spectrum function [78] [80]. The common characteristic frequency of a functional group of K proteins can be computed by the cross-spectral function defined in (4.1).

$$S(w) = |X_1(w)|\,|X_2(w)|\,|X_3(w)|\cdots|X_K(w)| \tag{4.1}$$

where $X_1(w)X_2(w)\cdots X_K(w)$ are the DFTs corresponding to the K proteins. The product of the amplitude spectra of the protein sequences as in Eq. (4.1) of a functional group is referred to as the consensus spectrum. Peak frequencies in the consensus spectrum denote the characteristic frequencies for all the proteins analyzed. It has been demonstrated that if a group of proteins has only one common function, then the consensus spectrum has one significant peak. If a protein performs more than one function, then each function corresponds to a unique characteristic frequency in the cross spectra. The numerical sequence (basic bovine and acidic bovine) and the consensus spectrum of the group of fibroblast growth factors (FGF) are shown in Figs. 4.2 and 4.3, respectively. This constitutes a family of proteins that affect the growth, differentiation and survival of certain cell. This particular function of the family is clearly shown as a peak at the normalized frequency of 0.90 in the consensus spectrum.

The RRM characteristic frequency in the consensus spectrum corresponds to a particular biological function of the family of proteins. Therefore, determination

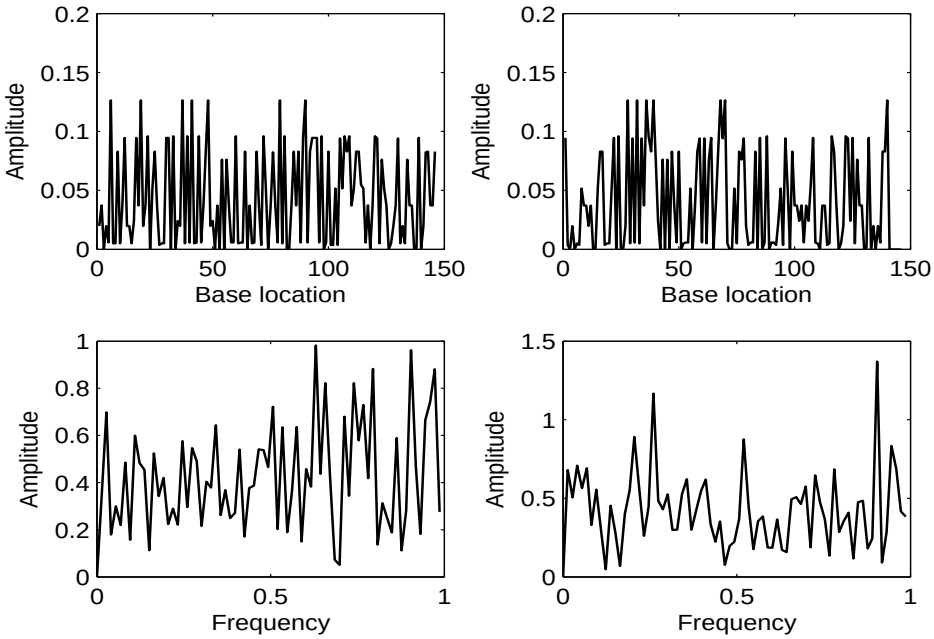

Figure 4.2: The numerical sequence and corresponding DFTs of the basic bovine (left) and acidic bovine (right)

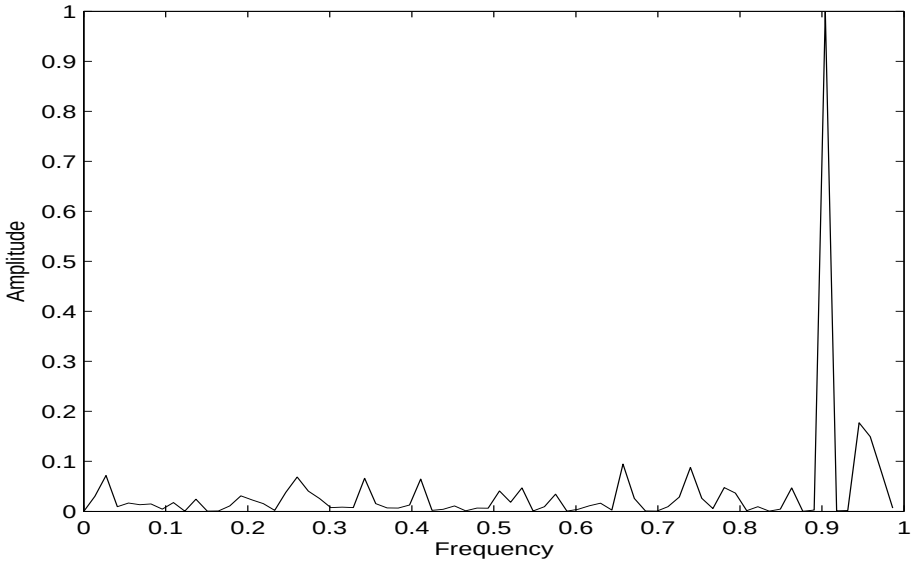

Figure 4.3: The consensus spectrum of the FGF family. The peak corresponds to the characteristic frequency relevant to a certain biological function

of the characteristic frequency enables identification of the individual amino acids i.e. the hot spots that contribute to it. A simple procedure has been adopted to identify the hot spots by altering the amplitude of the Fourier coefficients corresponding to the characteristic frequencies. It determines those amino acids which are most affected by the changes in the amplitude that belong to the characteristic frequency. The difficulty in this method is that a change in one Fourier coefficient affects all the samples in the numerical sequence of the protein, thereby provides an unreliable result. As the spectrum of the protein contains more frequency components along with the characteristic frequency, it confirms that the characteristics of the signal changes throughout the samples i.e. non-stationary in nature. A joint time-frequency analysis is needed for analyzing the change of the characteristic frequency in this case. This issue is resolved in this chapter by using the S-transform which is a better candidate for time-frequency analysis. Therefore a new method of time-frequency filtering using the S-transform has been proposed as a promising method to identify the amino acids (hot spots) corresponding to the characteristic frequencies.

4.4 Time-frequency analysis

By measuring and processing the genomic signals in time domain and frequency domain alone do not provide more information regarding the structure, sequence and pattern of the molecules. Hence a joint time-frequency analysis based approach has been evolved which provides a better understanding of the hidden artifacts in genomic signals. Time-frequency representations describe signals in terms of their joint time and frequency content [9]. These representations are useful for analyzing signals with both time and frequency variations such as speech, music, biomedical signal [15] and geophysical signals [17]. Time-frequency analysis is particularly useful for analyzing signals with continuously time-varying frequency content i.e. non-stationary signals. Many approaches to time-frequency analysis have been widely used for a number of years. Time-frequency analysis (TFA) is of

great interest when the signal models are unavailable. In those cases, the time or the frequency domain descriptions of a signal alone cannot provide comprehensive information for pattern identification and classification [10]. The time domain lacks the frequency description of the signals. The Fourier transform of the signal cannot depict how the spectral content of the signal changes with time, which is critical in many non-stationary signals in practice. Hence the time variable is introduced in the Fourier based analysis in order to provide a proper description of the spectral changes as a function of time. Hence the TFA evaluates the energy concentration along the frequency axis at a given time instant and thus provides a joint time-frequency representation of the signal. The S-transform is an excellent time-frequency analysis technique, which enjoys the advantage of both STFT and wavelet transform.
The localization potentiality of S-transform has been dealt in section 2.2.3 of chapter 2. Further, the new filtering approach using S-transform has been described in section 3.5 of chapter 3. These two characteristics of S-transform is used in this chapter for achieving improved identification of hot spots in proteins.

4.5 Hot spot localization in proteins using the proposed S-transform filtering approach

In order to make suitable the S-transform to apply on the proteins, it needs to be converted to a numerical sequences. The amino acids are assigned by the corresponding EIIP value which ranges from 0 to 0.1263, thereby provides a DC bias (average value) in the numerical sequence of the protein. This is an offset to the signal that has no meaning in the context of spectrum analysis. Hence the DC bias may mislead the peaks in the spectrum of the protein which has to be removed before computing the Fourier transform. Then the consensus spectrum is evaluated using Eq. (4.1) and the peak in the spectrum corresponds to the characteristic frequency of the family of proteins. The difficulty in the consensus spectrum is that it is unable to identify the individual amino acids which contribute to the characteristic peak frequency. Thus higher domain time-frequency analysis has been

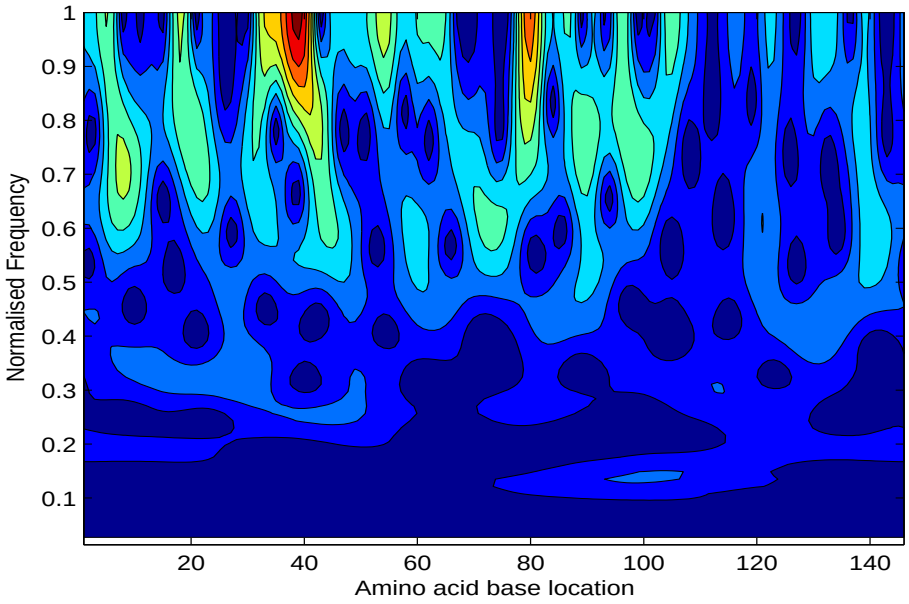

Figure 4.4: The contour plot of the spectrum of basic bovine FGF protein using S-transform. The high intensity color regions in the spectrum correspond to the characteristic frquency.

used for this purpose. The spectrum of the protein under consideration is calculated to observe the distribution of the energy of the characteristic frequency throughout the sequence. For illustration the spectrum of basic bovine FGF is obtained by the S-transform method is shown in Fig.4.4.

The S-transform spectrum shows high energy regions in the protein sequence corresponding to the characteristic frequency. In addition, it exhibits a number of insignificant frequencies in the spectrum along with the characteristic frequency. In order to reduce the noisy frequencies and to boost up the energy at the characteristic frequency, the consensus spectrum is multiplied with the S-transform spectrum for each sample. The S-transform spectrum of basic bovine protein after multiplication of the consensus spectrum is shown in Fig. 4.5. The distribution of energy of the characteristic frequency which has relevance to the biological function is shown in the plot. It provides distinct energy concentrated areas in the time-frequency plane where that characteristic frequency is dominant. Then a specific band limited

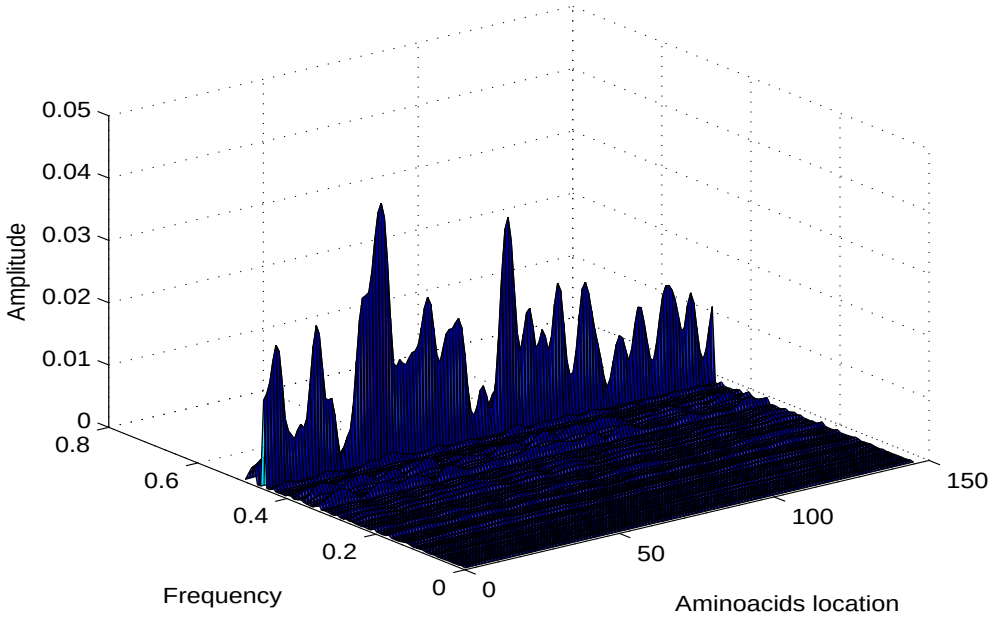

Figure 4.5: The surface plot of S-transform spectrum of the basic bovine FGF after multiplication with the consensus spectrum

time-frequency filter as suggested in section 3.5 of chapter 2 is designed to separate the frequency of interest (characteristic frequency). The filtered signal contains only the characteristic frequency which corresponds to the hotspots. Hence, the hot psots are identified by thresholding the energy of the filtered signal. The whole process of S-transform based filtering approach for hot spot identification is depicted in a flow chart as shown in Fig. 4.6.

The complete step-by- step procedure of the proposed approach is described as follows:

1. Convert the protein sequences belonging to the functional group of interest into numerical sequences using the EIIP values (Table 4.1).

2. Compute the DFTs of the numerical sequences and their consensus spectrum to determine their characteristic frequency.

3. Compute the spectrum of the protein sequence of interest using the

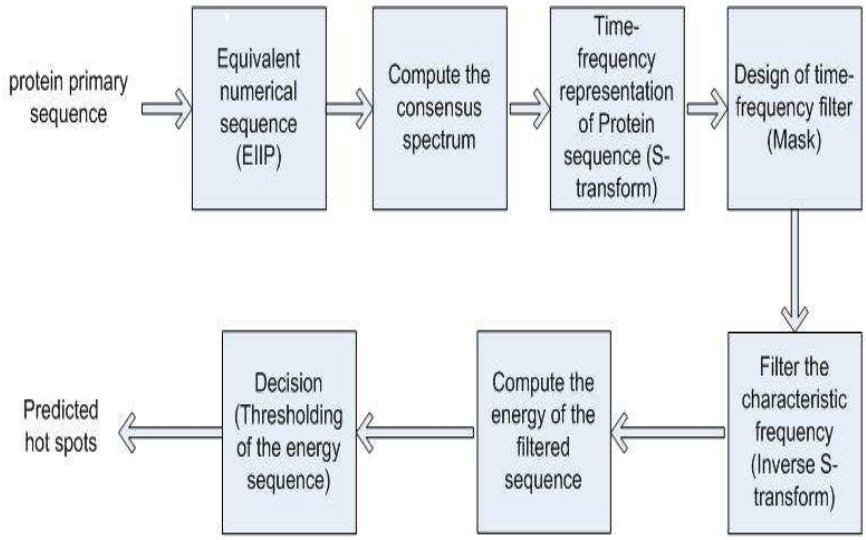

Figure 4.6: The flow chart of S-transform based filtering approach for hot spot identification

S-transform.

4. Multiply the S-transform Spectrum with the consensus spectrum in each time sample instant to supress the unwanted noise frequencies.

5. Design the band limited filter in time-frequency domain which selects the characteristic frequency and activates during the specific regions in the time-frequency plane.

6. Filter the protein numerical sequence of interest by using the time-frequency filter.

The peaks in the energy of the filtered output signal identify the locations of the hot spots [83]. If the output signal is denoted as $y(n)$, then its energy is given by

$$E(n) = |y(n)|^2 \tag{4.2}$$

This energy is referred to as the energy sequence corresponding to a protein at the characteristic frequency.

4.6 Performance analysis of the proposed approach

The potentiality of the proposed method is illustrated by using a set of 10 proteins from different functional family obtained from the standard databases. In the dataset each protein has no significance sequence similarity to any other protein. The sequence length, characteristic frequency and PDB ID of the corresponding protein are listed in Table 4.2. The detailed proeins in the same functional group used for the computation of consensus spectrum are also provided in Table 4.3. There are many freely available databases like protein data bank (PDB) [87], Swiss-Port [88] etc., where the primary sequence of the proteins are available. These databases are reliable and strongly recommended by the biological community. Protein hot spot location data obtained through alanine-scanning mutagenesis (ASM) have been compiled into an online database named the alanine scanning energetics database (ASEdb) [84]. This is a standard repository for hot spot location data used and maintained by the biological community. Each residue in the database is considered as hot spot if its corresponding $\Delta\Delta G$ is equal or higher than 2.0 kcal/mol. On the other hand the computational Robetta interface alanine scanning (Robetta-Ala) [66] [67] is another method of hot spot prediction which employs the residue's $\Delta\Delta G$ more than 1 kcal/mol as threshold to predict the hot spots. These two are well known energy based methods and have been used for the identification purpose. Hence both the ASEdb and Robetta-Ala database has used as a benchmark to compare all the hot spots identified by the proposed method.

4.6.1 Evaluation criteria

The hot spots in the protein sequence can be determined by comparing the energy at the regions corresponding to characteristic frequency to a reference energy level

Table 4.2: The protein sequences investigated

Organism	Protein name	PDB ID	Sequence length	Characteristic frequency
Human	Fibroblast Growth Factor	4fgf	146	0.904
C.fimi	Endoglucanase C	1ulo	152	0.093
Bacteria	Trap	1wap	75	0.247
Human	Human alpha hemoglobin	1vwt	142	0.023
Human	Human Growth Hormone (HGH)	3hhr	190	0.270
Bacteria	Barstar	1brs	89	0.321
Bacteria	Barnase	1brs	110	0.321
Human	Interleukin-4 (IL4)	1rcb	129	0.587
E.Coli	Colicin-E9 Immunity (IM9)	1bxi	86	0.190
Human	Human growth hormone binding (HGHbp)	3hhr	203	0.270

which governs the resolution of the method. First energy at the characteristic frequency is computed using Eq. (4.2) which gives rise to peaks at certain regions where it is dominant in protein sequence. Then the average energy of the filtered output is computed which is used as a reference level for indicating the hot spots in protein sequence. The ratio of the energy at the peaks of the filtered sequence to that average value is set as threshold (t_p) criteria for identifying the hot spots. If t_p is 1, the threshold is same as the average value, then the energy peaks which are more than that is considered as hot spots. The efficiency of the method in identifying the hot spots depends on the threshold value. Hence the t_p value acts as a parameter to control the resolution of the methods used.

4.6.2 Experimental study

To demonstrate the capability of the proposed method, the human basic bovine FGF protein is used for the analysis and the identified hot spots are shown in Fig. 4.7. The peaks correspond to the hot spot locations. These locations are identified by putting a threshold in the energy sequence. The average energy as the threshold

Table 4.3: Proteins of functional family used for computation of consensus spectrum

Protein name	Swiss-Port ID
Fibroblast Growth Factor	P05230,P09038,P15656,P55075,O15520, O54769,Q9EPC2,Q9HCT0,Q9QY10
Endoglucanase C	P0C2S3,P14090,P19570,P27033,P37699, P38534,Q93GB3,A3DJ77
Trap	P19466,P48064,Q2RHB9,Q8EQB3, C5D3E7,Q9x6J6
Human alpha hemoglobin	P60524,P01958,P02062,P69905,P68871,P68050, P01942,P01946,P68048
Human Growth Hormone (HGH)	P10912,P16310,P16882,P19941,Q9JI97, Q9TU69,Q02092,O46600
Barstar	P11540,A7FDT9,A9R1V5,B4TJT7,B5pWB6, C5BAW5,C7MPS8,Q2SZB1,Q62H00
Barnase	P10912,P00648,D0KFB0,C9NF27,C6XRM1, C6UF64,C4ZK78,B7M0V1
Interleukin-4 (IL4)	P05112,P07750,P16382,P20096,P30368,P42202, P51744,Q8HYB1,Q04745
Colicin-E9 Immunity (IM9)	P13479,P15176,B9VMA0
Human growth hormone binding	P79108,P79194,Q9XSZ1,Q95JF2,Q95ML5, Q28575

value is shown by a dotted line. A threshold of 90 percentile of the average energy is used to locate the hot spots in all the proteins studied. Hence the actual hot spots for the ten proteins identified by the proposed method are calculated and compared with those obtained by the digital filtering technique and also with the alanine scan compiled from both the ASEdb and Robetta-Ala. The hot spots are listed in Table 4.4. It reveals that the proposed method identifies 79% of the total hotspots of the proteins investigated which is 67% in digital filtering method. In this study the hot spots obtained from the alanine scans (both ASEdb and Robetta-Ala) have used as the reference for the comparison. For the detailed study of classification of hot spots the receiver operating characteristic (ROC) analysis is used. The ROC curve represents the trade off between the true positive rate (TPR) and the false positive rate (FPR) achieved by the predictors for varying threhold values on the

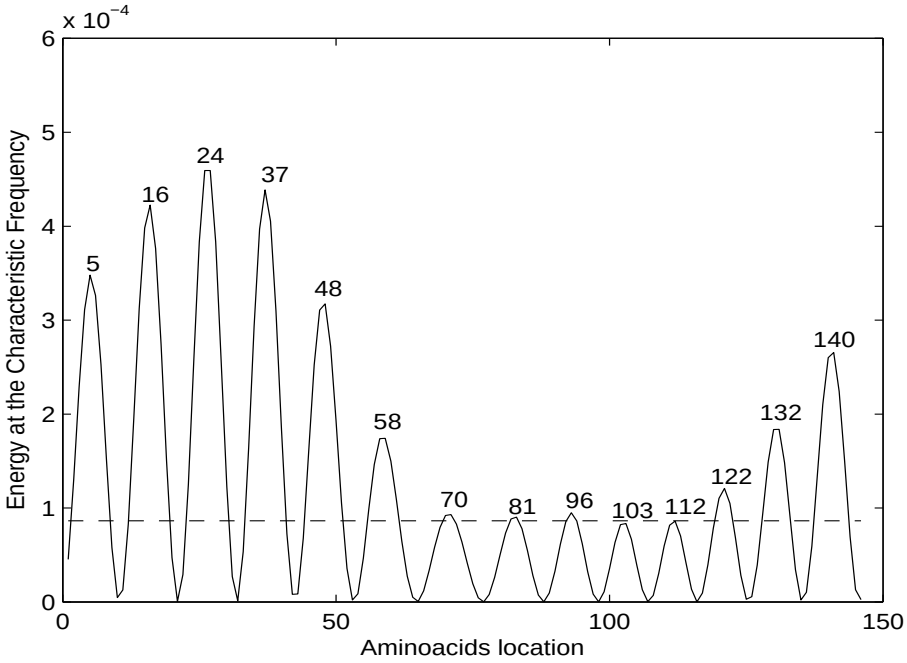

Figure 4.7: Hot spot locations of Human basic bovine FGF protein

average energy of the filtered sequence. These curves for the two methods are shown in Fig. 4.8. We have also computed the performance measures such as sensitivity (S_n), specificity (S_p), positive predictive value (PPV), negative predictive value (NPV), area under the curve, (AUC) etc. for both the methods and are presented in Table 4.5. It is clear that the proposed method provides superior performance over the frequency domain digital filtering technique in identifying the hot spots. Some more locations are also identified by the proposed scheme as false positive which are considered as probable hot spots and are repoted in Table 4.6.

A comparison study of the results of the proposed scheme with the other existing computational methods [68]- [74] has been done in order to calculate the efficiency of the proposed method. These computational methods use the knowledge of ASEdb database for the prediction of the hot spots. In order to have a common platform to compare the performance of these methods with the proposed signal processing method, the ASEdb has been used as a standard and used a subset of the dataset containing five proteins forming complexes (hGH, hGHbp, Barnase, Barstar and

Table 4.4: Comparison study of hot spots identification in proteins by the proposed method and digital filtering approach

Protein name	Alanine scan (ASEdb+Robetta)	Digital filtering	S-Transform Filtering
Fibroblast Growth Factor	24,96,103,140	24,26	24,96,103,140
Endoglucanase C	19,50,84	50	50,84
TRAP	37,40,56,58	37,40,56	40,56,59
Human Alpha Hemoglobin	18,22,36, 43,59	37,60	22,36,60
Interleukin-4	9,88	9,88	9,88
Human Growth Hormone	18,25,42,45,46,64, 168,171,172,175, 178,179	26,41,45,64,168, 171,175,178,179	18,25,42,47, 65, 168,172,175,178, 180
Human Growth Hormone binding	43,76,104,105, 127,165,169	43,105,127, 164,165,169	43,103,105, 127,165,170
Barnase	27,58,59,60, 73,87,102	27,59,73,87, 102	27,58,60,73, 86,102
Barstar	29,35,39,42, 76	35,38,42	29,35,38,42
Colicin-E9	30,33,34,38, 41,50,51,55	34,41,50,51, 55	33,41,50,51 55

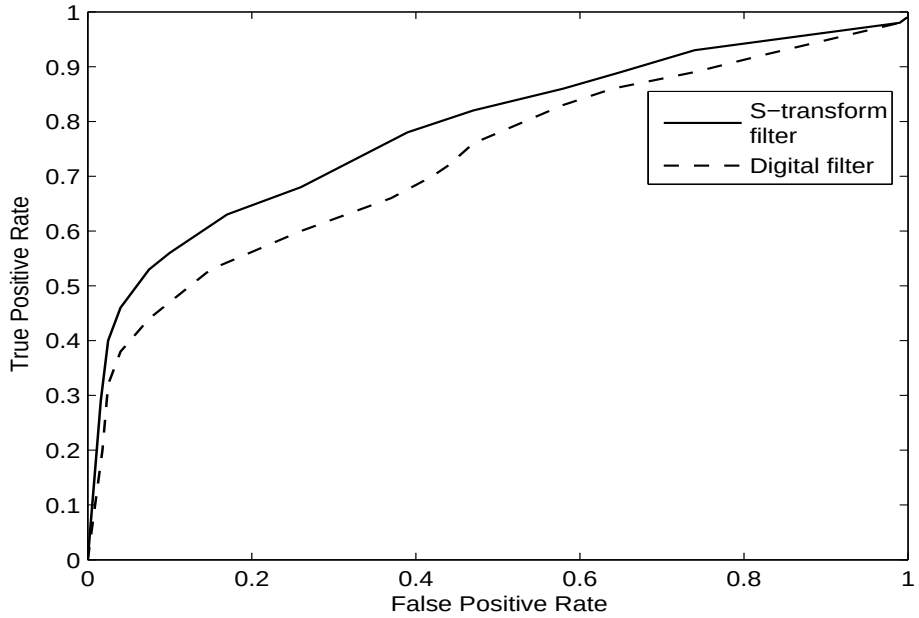

Figure 4.8: ROC curve comparison of the proposed method and Digital filtering method

Table 4.5: Performance evaluation of S-transform and digital filtering approaches for hot spot identification

performance evaluation	S-transform filtering	Digital filtering
S_n	79%	67%
S_p	59%	57%
PPV	52%	46%
NPV	84%	76%
Average success rate	67%	60%
AUC	0.7762	0.7302

$S_n = \frac{TP}{TP+FN}$, $S_p = \frac{TN}{FP+TN}$, $PPV = \frac{TP}{TP+FP}$,$NPV = \frac{TN}{FN+TN}$, Average success rate= $\frac{TP+TN}{TP+FP+FN+TN}$

Table 4.6: The newly identified hot spots by the proposed S-transform filtering method

Protein name	Newly identified hot spots
Fibroblast Growth Factor	6,16,37,48,58,70,81,112,122,132
Endoglucanase C	66,139
Trap	4,7,12,17,47,65,70
Human alpha hemoglobin	76,131
Human Growth Hormone	59,70,74,77,81,85,89,92,96,100, 103,107,147,150,154,158,161,184
Barstar	4,8,32,45,48,51,55,58,65
Barnase	3,6,9,12,20,65,69,79,82,89,92,96,99
Interleukin-4(IL4)	3,32,35,54,59,61,64,68,71,73, 96,98,103,105,118,121,123,125
Colicin-E9 Immunity(IM9)	4,62,68,73,79,84
Human growth hormone binding	2,6,10,14,17,22,26,30,34,38,45,51,55,59,62,66, 85,89,93,113,116,133,136,140,144,147,151, 155,159,175,180,183,187,191,195

Table 4.7: Comparison study of hot spots identification in proteins by different computational methods

Methods	HGH	HGHbp	Barnase	Barstar	IM9
ASEdb [84]	172,175,176, 178	43,104,105, 165,169	27,58,59, 73,87,102	29,35,39	33,34,41, 50,51,55
Robetta-Ala [66]	18,25,42,45,46,64, 168,171,175,179	43,76,104, 127,169	27,59,60, 87,102	29,35,39 42,76	30,33,38, 50,55
Digital filtering [83]	26,41,45,64,168, 171,175,178,179	43,105,127, 164,165,169	27,59,73, 87,102	35,38,42	34,41,50, 51,55
S-transform filtering	18,25,42,47, 65, 168,172,175, 178,180	43,103,105, 127,165,170	27,58,60, 73,86,102	29,35,38, 42	33,41,50, 51,55
KFC Server [71]	41,42,61,62,67, 167,168,171,172, 174,175,178,182, 189	43,44,76,101, 102,104,105, 123,164,165 ,169	27,56,58, 59,83,87, 102	29,35,39	30,33,34, 41,47,49, 50,51,53, 54,55,62
Hotsprint [72]	21,41,61,67,166, 167,170,171,173, 174,178,181,188	44,99,100,102, 162,163,166, 167,168	27,35,56, 58,60,83, 87,102,103	27,30,31, 33,34,35, 36,39,42	47,51,53, 55,56
HotPOINT [74]	25,41,45,67,164, 171,174,175,178, 179,182,189	43,76,103,104, 108,122,123, 169,170	35,56,83, 87,102, 103	30,34,35, 38,39	23,25,30, 33,34,37, 50,53,54
ISIS [69]	18,25,46	43,104,165,169	59,60,73	29	50

Table 4.8: Comparison of performance (in percentage) of different computational methods

Methods	S_n	S_p	PPV	NPV	Average accuracy
S-Transform filtering	83.33	84.80	62.50	94.37	84.47
Digital Filtering	79.17	79.75	54.29	92.65	79.61
KFC Server	79.17	83.50	59.38	92.96	82.52
Hotsprint	58.33	86.08	56.00	87.18	79.61
HotPOINT	58.33	81.01	48.28	86.49	75.73
ISIS	33.42	67.00	32.53	76.81	59.22

Colicin E-9) for the analysis. Total 103 experimental alanine mutations from the dimers have used for the study, out of which 39 residues are hot spots and rest are non hot spot residues. The hot spots identified by these methods are presented in Table 4.7 and the performance comparison is also repoted in Table 4.8. Table 4.8 clearly shows the superiority of our proposed approach over its counterparts as it provides the best accuracy in predicting the hot spots.

4.7 Discussion of results

The identification of hot spots in protein by the proposed method is validated by comparing the results with that obtained from biological methods like the alanine scanning mutagenesis (ASM). Further the hot spots prediction of the proteins has been analyzed in relation to the 3D structure. For the illustration purpose the 3D structure of the barnase-barstar complex (PDB ID:1brs) is shown in Fig. 4.9. Barnase is green and barstar is sky-blue in color. The detected true hot spots are marked as red spacefill for barnase and yellow spacefill for barstar. Similarly, the probable hot spots are marked as red and yellow sticks for both the proteins respectively. From Fig. 4.9, it has been seen that the detected true hot spots are located at the interface region to the neighbour protein. Among the probable ones some are located at the spatial vicinity of these true hot spots (Gly:65, Arg:69, Thr:99 in barnase and Glu:32 val:45 in barstar) and are also interface residues which may provide the structural scaffold of the interface and thus needs further investigation. The remaining probable hotspots are placed far away from the faced region, but falsely identified as hot spots. These possible hot spots may provide some insight that may ameliorate the underlying functioning of the proteins. Although the proposed S-transform based filtering method identifies the hot spots in a better way, it also predicts some false positives. The limitations of this approach is that it provides low frequency resolution at higher frequencies and low time resolution at lower frequencies. At higher frequencies the signature of the events smears in frequency direction where as in lower frequencies it smears in time direction. This

basically happens due to the scaling nature of the Gaussian window during spectrum computation. Thus, it affects the time-frequency filtering operation which may lead to the false positives in the prediction. Improving the resolution of the spectrum and efficiently designing the mask in time-frequency plane can further be reduced the false positives in the prediction. Computational prediction of the hot spots

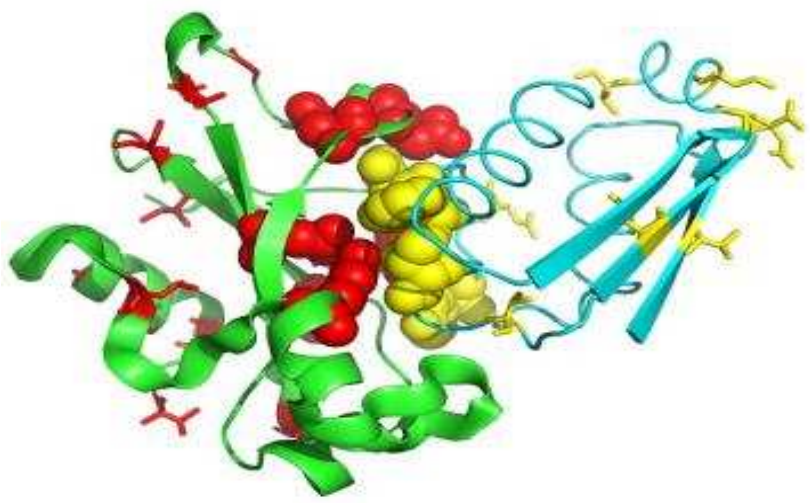

Figure 4.9: The 3D structure of barnase-barstar complex (PDB ID:1brs) showing the hot spots. The green one is barnase and sky-blue one is barstar. The true hot spots are marked as spheres and the sticks represents the predicted probable hots pots

provides a platform for the analysis of protein target interactions. Thus it saves time, effort and cost for the identification of hot spots in protein. Generally the existing computational methods for hot spot identification are based on the knowledge of interface regions in the protein complex and thus the predictions are limited to the interface residues only. But the proposed signal processing technique does not need a prior 3D structural information of the protein for the localization of hot spots. Thus it helps in identifying those sites in a newly discovered protein whose primary

sequence information only is available.

In-depth study of the nature of protein interactions would further facilitate development of various new effective drugs and molecular medicines. It would help in designing peptides with desired spectral and functional characteristics. The computational identification of the new hot spots by our approach would also help in the experimental process of effective mutation.

4.8 Conclusion

In this chapter, a new approach for the identification of the hot spots in protein using S-transform based filtering has been developed. The effectiveness and accuracy of the proposed method are evaluated by taking some protein sequences and the results are compared with those obtained by other existing methods. The results have demonstrated that the proposed method provides improved identification capacity of hot spots compared to the digital filtering and other existing methods. In addition, the new approach has identified some unknown locations of hot spots which need further analysis and investigation. As the proposed method do not use the knowledge of the structure of protein complex, hence it can be effectively employed in hot spot prediction where the interface region is unknown.

Chapter 5

A Novel Feature Representation based Classification of Protein Structural Class

Chapter 5

A Novel Feature Representation based Classification of Protein Structural Class

5.1 Introduction

In the post genomic era, study of sequence to structure relationship and functional annotation plays an important role in molecular biology. In this context the protein fold prediction is one of the major problems in protein science. The structural class has become one of the most important features for characterizing the overall folding type of a protein and has played an important role in rational drug design, pharmacology and many other applications [89]. The functions of protein are relevant to its 3D structure and can be efficiently determined by the sequence and structure analysis [90–92]. The knowledge of protein structural class provides useful information towards the determination of protein structure. The exponential growth of newly discovered protein sequences by different scientific community has made a large gap between the number of sequence-known and the number of structure-known proteins. Hence, there exists a critical challenge to develop automated methods for fast and accurate determination of the structures of proteins in order to reduce the gap. Therefore, there is a need to develop computational methods for identifying the structural classes of newly found proteins based on their primary sequence.

The concept of protein structural classes was reported by Levitt and Chothia [93]

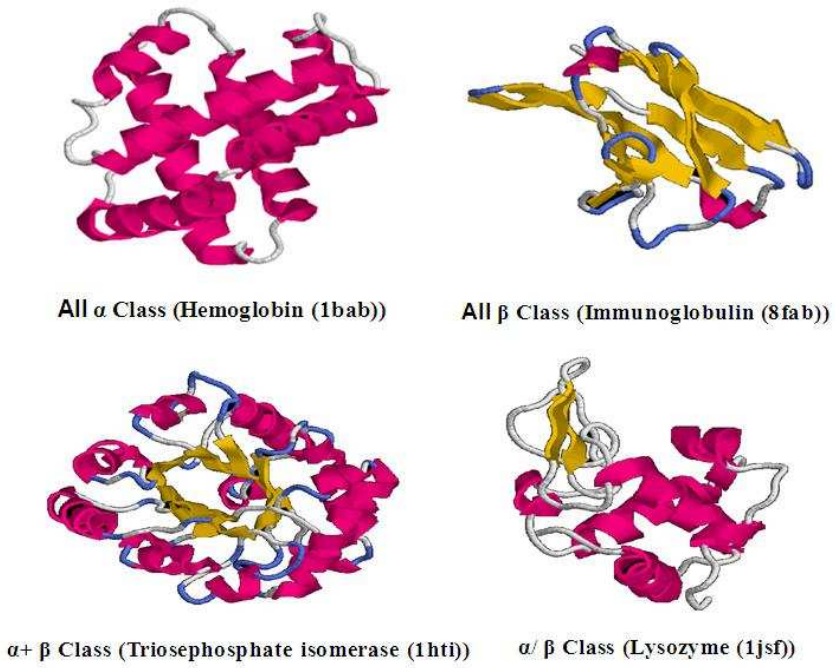

Figure 5.1: The four structural classes of protein

on a visual inspection of polypeptide chain topologies in a dataset of 31 globular proteins. They have proposed ten structural classes, four principal and six small classes of protein structures. But the biological community follows the first four principal classes, which are: all-α, all-β, $\alpha + \beta$ and α/β. The all-α and all-β classes represent structures that consist of mainly α-helices and β-strands, respectively. The α/β and $\alpha + \beta$ classes contain both α-helices and β-strands which are mainly interspersed and segregated. The α class proteins contain more than 45% α-helices and less than 5% β-strands. The β class proteins comprise of less than 5% α-helices and more than 45% β-strands. The $\alpha + \beta$ class proteins contain more than 30% α-helices and more than 20% β-strands with dominantly anti-parallel β-strands. The α/β class proteins contain more than 30% α-helices and more than 20% β-strands,

with dominantly parallel β-strands. These class definitions are well accepted and are still commonly used by many researchers. A pictorial view of the four structural classes of protein is shown in Fig. 5.1.

5.1.1 Review of protein structural class prediction

The problem of predicting protein structural classes from the primary sequence is mainly focused on two aspects. The first one is effective representation of the protein sequence and the second one is the development of the powerful classification algorithms to efficiently predict the desired class. Many in-silico structural class prediction algorithms and methods have been proposed in the last few decades. During this period many amino acid indices and features are used for the assignment of the protein sequence. Nakashima *et al.* [94] have indicated that the protein structural classes are strongly related to amino acid composition (AAC). Later on auto-correlation functions based on non-bonded residue energy, polypeptide composition [95] and complexity measure factor [96] have been used by many researchers. Subsequently several classification methods such as distance classifier [97] [98], component coupled methods [99], principal component analysis [97], Bayesian classifier, fuzzy clustering [100] [101], neural network [102] [103], rough sets [104] and support vector machines [105] [106] [107] have been suggested in the literature. Although promising results have been achieved in many cases, the representation of protein with the AAC lacks the sequence-order and sequence-length information [108] [109]. Though the amino acid composition pattern of a protein is closely related to its cell attributes, it is unable to distinguish between two protein sequences with the same AAC, but different orders of arrangement. Hence, the sequence order effect should not be ignored as a factor relating to protein structure [110]. Chou introduced a new concept of pseudo amino acid composition (PseAAC) [109] [111], in which both the sequence order and length information of the protein sequence have been considered for the representation of the protein and is used to predict various attributes of it. The introduction of PseAAC has greatly

stimulated the development of protein structural class prediction. Various variants of the PseAAC have also been also reported. Many other information regarding the protein sequence has been embedded in the PseAAC to optimally reflect the sequence order and length effects. Zhan Li *et al.* [112] have incorporated the wavelet power spectrum into the PseAAC to reflect the long range interaction of amino acids in the protein. Hui Liu et al. [113] [114] have used the Fourier spectrum analysis with the PseAAC to improve the membrane protein prediction. Basically the objective of all these representations is to form a discrete model to predict various attributes of protein. In this chapter a novel method of PseAAC has been proposed by suitably embedding the amino acid composition information, the amphiphilic correlation factors and the spectral characteristics of the protein. The proposed feature vector is expected to reflect the sequence pattern information relevant to the structure of the protein.

5.2 Feature representation method of protein

5.2.1 Amino acid composition (AAC) feature of protein

In this form of representation, each protein is defined by a 20-dimensional feature vector in Euclidean space. The protein corresponds to a point whose co-ordinates are given by the occurrence frequencies of the 20 constituent amino acids.

For a query protein x, let $f_i(x)(i = 1, 2, \cdots, 20)$ represents the occurrence frequencies of its 20 constituent amino acids. Hence, the composition of the amino acids (p_k) in the query protein is given by

$$p_k(x) = \frac{f_k(x)}{\sum_{i=1}^{20} f_i(x)}, i, k = 1, 2, \cdots, 20 \tag{5.1}$$

The protein x in the composition space is defined as

$$P(x) = [p_1(x), p_2(x), \cdots, p_{20}(x)] \tag{5.2}$$

In this type of representation, the protein sequence order and length information are completely lost which in turn affects the prediction accuracy.

5.2.2 Amphiphilic Pseudo amino acid composition (AmPseAAC) feature of protein

To include all the details of its sequence order and length, the sample of a protein must be represented by its entire sequence. Unfortunately, it is not feasible to establish a predictor with such a requirement, as it requires huge experiments. Further, the lengths of protein sequences vary widely, which pose an additional difficulty for admitting the sequence-order information in the feature extraction of protein [116]. To alleviate this problem Chou [111] has proposed an effective way of representation of protein known as pseudo amino acid composition (PseAAC). In this representation, the protein character sequence is coded by some of its physicochemical properties. The hydrophobicity and hydrophilicity of the constituent amino acids in a protein play very important role on its folding, its interaction with the environment and other molecules, as well as its catalytic mechanism [117]. Thus these two indices may be used to effectively reflect the sequence order effects. Different types of proteins have different amphiphilic features, corresponding to different hydrophobic and hydrophilic order patterns. Thus, the sequence-order information of protein can be derived quite effectively as follows:

Suppose a protein P with a sequence of L amino acid residues is defined as

$$P_1 P_2 P_3 \cdots\cdots\cdots\cdots P_L$$

where P_1 represents the residue at position 1 along the sequence, P_2 the residue at position 2 and so forth. The sequence order effect along a protein chain is approximately reflected by a set of sequence order correlation factors defined as

$$\theta_\tau = \frac{1}{L-\tau}\sum_{i=1}^{L-\tau}\Theta(P_i, P_{i+\tau}), (\tau = 1, 2, \cdots, \lambda \quad and \quad \lambda < L) \tag{5.3}$$

In Eq. (5.3), L and θ_τ denote the length of the protein and the τ th rank of coupling factor that harbors the τ th sequence order correlation factor respectively. The correlation function $\Theta(P_i, P_j)$ may assume different forms of representation.

The $\Theta(P_i, P_j)$ term is defined as

$$\Theta(P_i, P_j) = H(P_i) \times H(P_j) \tag{5.4}$$

where $H(P_i)$ and $H(P_j)$ represent hydrophobicity values of the amino acids P_i and P_j, respectively. Similarly the correlation factors for hydrophilicity values are also calculated. These two types of correlation factors form the basis of amphiphilic pseudo AAC (AmPseAAC) feature vector of a protein which have been successfully employed for predicting the enzyme subfamily classes and membrane protein types [110] [113]. The hydrophobicity and hydrophilicity values of the amino acids defined by Tanford [118] and Hopp & Woods [119] are used in this study. These values are listed in Table 5.1. In Eq. (5.3), θ_1 is called the first tier correlation factors that reflect the sequence order correlation between the most contiguous residues along the protein sequence through hydrophobicity and hydrophilicity. θ_2 corresponds to the second tier correlation factors that reflect the sequence order correlation between all the second most contiguous residues and so forth. Before substituting the hydrophobicity and hydrophilicity values in Eq. (5.4), these are subjected to a standard conversion. The objective of the conversion is to make the coded sequence as zero mean over the 20 native amino acids. It remains unchanged if it undergoes through the same conversion procedure again. Hence a considerable amount of sequence order information has been incorporated into the 2λ correlation factors through the amphiphilic values of the amino acid residues along a protein chain.

5.2.3 Spectrum based feature of protein

The key idea in this study is to establish a powerful identifier that can catch their characteristic sequence patterns for different structural classes. Primary structure of proteins occasionally shows periodic pattern of hydrophobicity. The periodicity in the hydrophobicity of amino acid sequence was studied for intrinsic membrane proteins [120]. As a result the frequency information of the sequence pattern are more effectively incorporated into a set of discrete components, and the prediction algorithms are used in a straight forward manner on such a formulation of protein

samples [113] [114]. The frequency information is collected by transferring the protein coded sequence to frequency domain. The goal of this spectral analysis is to identify the distribution of the power contained in a signal over the frequencies. The DFT is a potential tool to transform the discrete protein sequence to its

Table 5.1: The Hydrophobicity and Hydrophilicity values of the amino acids

Amino acid	Hydrophobicity	Hydrophilicity
Ala (A)	0.62	-0.5
Cys (C)	0.29	-1.0
Asp (D)	-0.90	3.0
Glu (E)	-0.74	3.0
Phe (F)	1.19	-2.5
Gly (G)	0.48	0.0
His (H)	-0.40	-0.5
Ile (I)	1.38	-1.8
Lys (K)	-1.50	3.0
Leu (L)	1.06	-1.8
Met (M)	0.64	-1.3
Asn (N)	-0.78	0.2
Pro (P)	0.12	0.0
Gln (Q)	-0.85	0.2
Arg (R)	-2.53	3.0
Ser (S)	-0.18	0.3
Thr (T)	-0.05	-0.4
Val (V)	1.08	-1.5
Trp (W)	0.81	-3.4
Tyr (Y)	0.26	-2.3

corresponding frequency domain. The DFT of the protein sequence (P) is defined as

$$X(k) = \sum_{n=1}^{L} H(p_n) e^{(\frac{-j2\pi nk}{L})}, k = 1, 2, \cdots\cdots, L \tag{5.5}$$

where $X(k)$ represents the periodicity features and the compositional pattern by sinusoidal waves with various frequencies. Therefore, the sequence order effect of the protein is partially reflected by the Fourier coefficients [113]. The amplitude

spectra contain the information about the signal and also exhibit the amino acids sequence order of a protein. The high frequency components are mostly due to noise and hence the low frequency components are more important [121]. This is similar to the case of protein internal motions where the low frequency components imply more biological functions [122] [123]. These dominant low frequency motions have a major effect on the structure (both alpha helix and beta sheets) formation of the protein. Hence the low frequency components are chosen as effective feature vector.

5.3 The proposed DCT amphiphilic pseudo amino acid composition feature representation scheme of protein

In this work, discrete cosine transform (DCT) [5] has been introduced as a suitable feature extractor of the complex Fourier coefficients. The DCT is a very well studied real valued technique and has been successfully used in variety of applications [6] [7]. Due to its useful properties, the DCT is chosen to be a better substitute of DFT in the context of feature extraction of protein sequences. A brief introduction of DCT is provided in chapter 2.

The low-frequency components of DCT represent the global information [122] [123] of the coded sequence. The type of structural class of protein is represented by the curve of the hydrophobic values of the residues whose global shape represented by the low-frequency components of the DCT. Hence, the low frequency DCT coefficients can be used to represent the spectral characteristics of the protein.

In the previous studies, the Fourier spectra of the discrete protein sequence have been incorporated into the Chou's pseudo amino acid composition process to form a feature vector [113] [114]. In many investigations the energy spectra of the correlation factors [121] [124] have also been used in the pseudo AAC method to represent a protein sample. Thus, both the correlation factors and the frequency spectra of the protein sequence have been employed in the pseudo AAC process to formulate a novel feature representation method. The correlation factors retain the

sequence order effect of the protein sequence and the low frequency coefficients of DCT preserves the global information of the protein sequence along with some of the order effect.Therefore, the AAC, the 2λ correlation factors of both hydrophobic and hydrophilic sequences and the δ low frequency DCT coefficients are embedded together to form the new pseudo amino acid composition vector, the DCTAmPseAAC. Accordingly, a protein sample is represented in the new PseAAC form as

$$P = \begin{bmatrix} p_1 \\ \vdots \\ p_{20} \\ p_{20+1} \\ \vdots \\ p_{20+\lambda} \\ p_{20+\lambda+1} \\ \vdots \\ p_{20+2\lambda} \\ p_{20+2\lambda+1} \\ \vdots \\ p_{20+2\lambda+\delta} \end{bmatrix}$$

where

$$p_u = \begin{cases} \frac{f_u}{\sum_{i=1}^{20} f_i + w \sum_{j=1}^{2\lambda} \theta_\tau + w \sum_{k=1}^{\delta} \gamma_k}, & (1 \leq u \leq 20) \\ \frac{w\theta_{u-20}}{\sum_{i=1}^{20} f_i + w \sum_{j=1}^{2\lambda} \theta_\tau + w \sum_{k=1}^{\delta} \gamma_k}, & (20+1 \leq u \leq 20+2\lambda) \\ \frac{w\gamma_{u-(20+2\lambda)}}{\sum_{i=1}^{20} f_i + w \sum_{j=1}^{2\lambda} \theta_\tau + w \sum_{k=1}^{\delta} \gamma_k}, & (20+2\lambda+1 \leq u \leq 20+2\lambda+\delta) \end{cases} \tag{5.6}$$

where $f_i, i = 1, 2, \cdots\cdots, 20$ are the normalized occurrence frequencies corresponding to 20 native amino acids in the protein P, the symbol θ_j represents the j-tier sequence correlation factor computed using (5.3) . The low frequency DCT coefficients of

the protein are denoted by γ_k and the symbol w represents the weight factor which governs the degree of the sequence order effect to be incorporated. The first 20 values in Eq. (5.6) represent the classic amino acid composition, the next 2λ values reflect the amphiphilic sequence correlation along the protein chain and the remaining δ discrete values contain the low frequency global information of the protein.

5.4 Classification strategy

In recent past, many statistical and machine learning algorithms have been applied to accurately predict the protein structural class. In this work, a kind of neural network classifier known as radial basis function neural network has been employed for classification. The RBF networks are suitable for solving function approximation and pattern classification problems [125] [25] because of their simple topological structure and their ability to learn in an explicit manner. In the classical RBF network, there is an input layer, a hidden layer consisting of nonlinear node function, an output layer and a set of weights to connect the hidden layer and output layer. The basis functions are usually chosen as Gaussian and the number of hidden units are fixed apriori using some properties of input data. The weights connecting the hidden units to the output layer are normally adapted following a least mean square algorithm. A brief introduction to radial basis function neural network has been discussed in chapter 2. The complete process of the feature representation and the class prediction is presented in Fig. 5.2.

5.5 Performance measures

Conventionally, in statistical prediction and classification problems, the prediction quality generally measured by three typical tests such as re-substitution test, independent datasets and jackknife test [91].

The re-substitution test is used to examine the self-consistency of a prediction model. During this process the class label of each protein in the database is predicted using

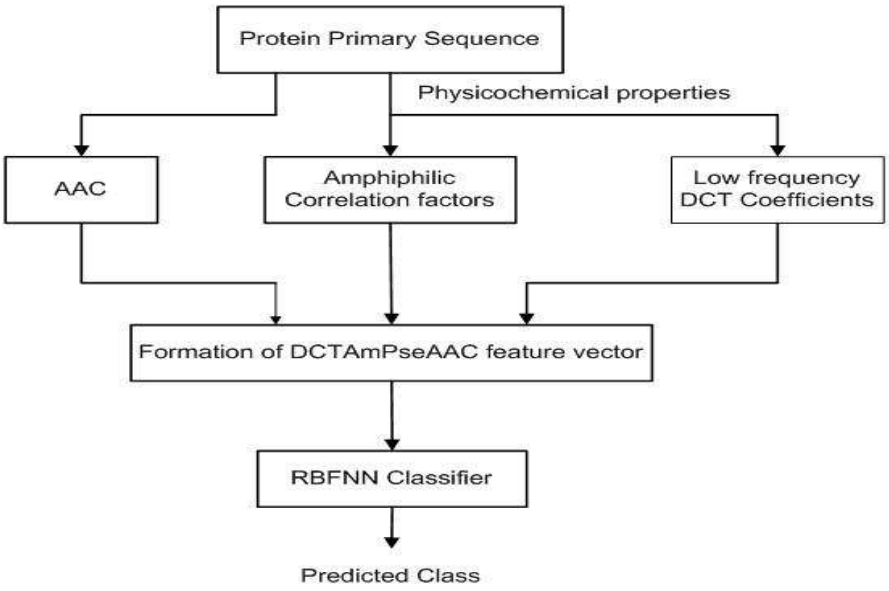

Figure 5.2: The flow graph of the proposed feature based classification approach

the rule parameters derived from the same dataset. This certainly overestimates the success rate of the model. In independent dataset test prediction is performed for a set of independent proteins, none of which is included in the training dataset.

To have a better generalizability performance of the predictor, a cross validation test is necessary. In this technique, the overall set of n training samples is randomly divided into m approximately equal size and balanced set of subsets. Then, each time one of these subsets is excluded from the overall training set and used as a test set. This process is repeated over the m subsets and the resultant test error rates are averaged to obtain the cross validation errors. When the subset size (m) is equal to n (size of the entire dataset), it is called leave-one-out cross validation (LOOCV) or Jackknife test. In Jackknife test one protein in the dataset is left out and the model is trained on the rest (n-1) proteins and tested on the left out sample. Then the sample is inserted back into the database and another protein is left out. This procedure is repeated until every protein in the database is left out for testing. The

Jackknife test is the most desired one and is a useful test used by most researchers to test the efficiency of the prediction models.

5.6 Results and Discussion

5.6.1 Datasets

In order to compare the efficiency of the proposed method in predicting the structural class of proteins, three standard data sets have been used. The first dataset constructed by Chou [98] contains 204 proteins. The average sequence similarity scores in the protein classes are 21% for all α, 30% for all β, 15% for α/β and 14% for $\alpha+\beta$ class. Hence there is no significant sequence similarity between the proteins in the dataset. Another two standard datasets constructed by Zhou [115] is also used in the present study which contains 277 and 498 protein domains respectively. All the three standard datasets have been used by many researchers in the class prediction methods. In these datasets the number of protein domains in each class is listed in Table 5.2.

Table 5.2: Benchmark datasets used for structural class prediction

Dataset	All α	All β	$\alpha+\beta$	α/β	Total
204 Domains	52	61	46	45	204
277 Domains	70	61	81	65	277
498 Domains	107	126	136	129	498

5.6.2 Experimental Results

To have a comparative performance study the proposed feature representation method is analyzed with many well studied classifiers such as neural network (multi layer perceptron), radial basis function network and linear discriminant analysis (LDA). The success rates of all the classifiers are evaluated with all the three benchmark datasets (204, 277 and 498 datasets). The results of the analysis are

listed in Table 5.3. From this table, it is evident that the RBF classifier yields best performance among all the classifiers and for all the three datasets. The average prediction accuracy of RBF classifier for the three datasets is 93.77 which is almost 5 to 20% higher than those obtained by other classifiers. The prediction accuracy associated with the 498 dataset is shown to be higher because it contains more similar/duplicate sequences. Among the four structural classes, $\alpha + \beta$ class is more difficult to predict as it has relatively large variability of helix and strand content for the protein as compared to other class. However, the proposed method also shows improved classification performance for the $\alpha + \beta$ class for all the datasets. Further

Table 5.3: Comparison of Jackknife classification accuracy (in percentage) of different classification algorithms using new (DCTAmPseAAC) feature representation method

Dataset	Algorithm	Jackknife Accuracy				
		All α	All β	$\alpha + \beta$	α/β	Overall
204 Domains	RBF	90.38	95.08	89.13	95.56	92.54
	MLP	90.38	93.44	73.91	86.67	86.76
	LDA	86.54	93.44	67.39	64.44	79.41
277 Domains	RBF	92.86	93.44	89.23	95.06	92.78
	MLP	90.00	88.52	78.46	88.89	86.64
	LDA	77.14	81.97	70.77	76.54	76.53
498 Domains	RBF	95.33	95.24	94.57	98.53	96.00
	MLP	89.72	93.65	88.37	91.91	90.96
	LDA	74.77	82.54	67.44	84.56	77.33

the effect of different features derived from the protein primary sequence have been examined on the classification accuracy. The classification accuracies of the AAC, AmPseAAC, and the proposed DCTAmPseAAC method are compared with the radial basis function network as the classifier. In case of AmPseAAC, empirical study showed that when λ is set to 10, best performance is achieved for all dataset. In case of DCT based methods best performance is also observed when δ is chosen to be 10. Hence 20 amino acid composition features, 20 amphiphilic correlation factors and 10 low frequency DCT components are embedded together to form the novel

DCTAmPseAAc feature vector. Thus total 50 features have been used to represent a protein sample. The weight factor (w) is chosen as 0.01 to normalize the feature vector. The comparative results of all the three representations are summarized in Table 5.4. The success rate of the proposed feature representation method is found to be the best as compared to those obtained by others. Hence, it is inferred that the proposed method captures features more relevant to the protein structure. Further the classification performance of the proposed feature representation with the RBF network is studied for the re-substitution and jackknife test. The results are presented in Table 5.5 for the three datasets. It is interesting to observe that for the re-substitution test it provides 100% accuracy for all the dataset and for Jackknife test, the classification accuracy is also higher.The histogram representation of the results of the proposed method is presented in annexure-II.

Table 5.4: Comparison of Jackknife classification accuracy (in percentage) using different feature representation methods

Dataset	Feature	Jackknife Accuracy				
		All α	All β	$\alpha+\beta$	α/β	Overall
204 Domains	AAC	84.62	93.44	82.61	86.67	87.25
	AmPseAAC	88.46	96.72	80.96	84.44	89.71
	DCTAmPseAAC	90.38	95.08	89.13	95.56	92.54
277 Domains	AAC	91.43	93.44	70.77	92.61	87.36
	AmPseAAC	92.86	95.08	73.85	93.83	89.17
	DCTAmPseAAC	92.86	93.44	89.23	95.06	92.78
498 Domains	AAC	91.59	93.65	89.15	94.85	92.37
	AmPseAAC	90.52	93.65	90.70	97.79	93.57
	DCTAmPseAAC	95.33	95.24	94.57	98.53	96.00

The performance of the new method is also compared with those obtained by recently reported prediction methods and the results are summarized in Tables 5.6-5.8. For the 204 dataset the classification accuracy of proposed scheme is compared with those obtained by the existing augmented covariant discriminant algorithm [126], fuzzy clustering [100], logitboost technique [127] [128], nearest

Table 5.5: Classification accuracy (in percentage) of the proposed (AmPseAAC+RBF) method for self-consistency and jackknife tests

Dataset	Test	Accuracy				
		All α	All β	$\alpha+\beta$	α/β	Overall
204 Domains	re-substitution	100	100	100	100	100
	jackknife	90.38	95.08	89.13	95.56	92.54
277 Domains	re-substitution	100	100	100	100	100
	jackknife	92.86	93.44	89.23	95.06	92.78
498 Domains	re-substitution	100	100	100	100	100
	jackknife	95.33	95.24	94.57	98.53	96.00

neighborhood network [112], distance based algorithms, support vector machine and its variants [129]. For the 277 and 498 datasets the comparison includes the results obtained from rough sets [104], neural network [130], component coupling algorithm, logitboost techniques [127] [128], nearest neighborhood network, VPMCD [131], support vector machines [132] and its variants. For the 204 dataset, the proposed scheme provides a little improvement (less than 1%) in the prediction as compared to the best results provided by the Fuzzy SVM and binary tree SVM. Another hybrid technique of genetic algorithm with SVM provides improved results than the proposed method, but it suffers from higher computational complexity. In case of the 277 dataset, the proposed method shows an improvement of at least 5% in the accuracy compared to the best performing PSI-BLAST collocated with SVM and also the SVM fusion method. Similarly, for the 498 dataset also it yields 1-2% improvement in the accuracy as compared to the existing best performing PSI-BLAST collocated with SVM, CWTPCA with SVM and logitboost techniques. All these test results demonstrate that the proposed feature based sequence representation method together with the RBF network based classifier provides best classification performance for all the benchmark datasets used. Further it may be noted that the radial basis function network with new proposed feature extraction method is very simple to implement and substantially provides improved

classification compared to those obtained by other reported classifiers.

5.7 Conclusion

In this chapter, a new promising feature representation method is presented by embedding the amino acid composition, the amphiphilic correlation factors and the low frequency DCT coefficients to represent a protein sample. The results of the Jackknife cross validation test using the standard datasets shows that the proposed method can be used as an efficient approach for predicting protein structural class. The present study also demonstrates that the composition of all the three features better reflects the overall sequence pattern of a protein than the individual one and enhance the success rate of prediction. The comparison study of the proposed scheme in association with a simple radial basis function network with the existing methods showed improvement of 2-5% using the standard datasets. The high success rate suggests that the proposed feature representation method can be used as a potential candidate for the protein structural class prediction and also for other related areas.

Table 5.6: Comparison of Jackknife accuracy (in percentage) of the best classifier that used the proposed feature and the other reported methods (for 204 Dataset)

Methods	Features used	Jackknife Accuracy				
		All α	All β	$\alpha+\beta$	α/β	Overall
Augmented covariant discriminant	PseAAC and Complexity measure factor	82.7	90.2	87	100	89.7
Unsupervised Fuzzy clustering	AAC	67.3	86.9	60.9	46.7	68.1
Supervised Fuzzy clustering	AAC	73.1	90.2	63.1	62.2	73.5
Logitboost	AAC	90.4	88.5	73.9	80.0	83.8
Binary Tree SVM	Pseudo AAC	90.4	100	97.8	73.9	91.2
SVM	Paired couple AAC	75	90	64	64	74.5
SVM	Pseudo AAC	88.5	96.7	73.9	77.8	85.3
SVM	PSI-BLAST based P-collocated AA pairs	90.4	100	93.5	91.1	94.1
Fuzzy SVM	Multi PseAAC	92.3	100	82.6	93.3	92.6
AAPCA	AAC	82	97	78	82	85
Euclidean distance	AAC	73	82	57	49	67
Hamming distance	AAC	71	89	57	49	68
Complexity distance measure	Conditional complexity measure	88.5	100	76.1	97.8	91.2
GASVM	Physical & structural PseAAC	100	100	90	97.8	99.5
RBFNN	**DCTAmPseAAC**	**90.38**	**95.08**	**89.13**	**95.56**	**92.54**

Table 5.7: Comparison of Jackknife accuracy (in percentage) of the best classifier that used the proposed feature and the other reported methods (for 277 Dataset)

Methods	Features used	Jackknife Accuracy				
		All α	All β	$\alpha+\beta$	α/β	Overall
Roughsets	AAC & Physicochemical properties	77.1	77.0	66.2	93.8	79.4
Component coupling	AAC	84.3	82.0	67.7	81.5	79.1
Neural network	AAC	68.6	85.2	56.9	86.4	74.7
SVM	AAC	74.3	82.2	72.3	87.7	79.4
Logitboost	AAC	81.4	88.5	72.3	92.6	84.1
SVM	PSI-BLAST based P-collocated AA pairs	91.2	91.4	76.9	93.4	87.7
SVM Fusion	Pseudo AAC	85.7	90.2	80.0	93.8	87.7
CWT with PCA	Pesudo AAC	85.7	90.2	80.1	87.7	85.9
VPMCD	AAC	85.73	85	84.4	92.7	84.2
MODWT with SVM	Physicochemical properties	86.96	88.52	66.15	88.89	82.97
Complexity distance with NN	complexity distance measure	91.4	83.6	69.2	93.8	85.2
GASVM	Physicochemical + structural features	84.3	88.5	70.7	92.6	84.5
RBFNN	**DCTAmPseAAC**	**92.86**	**93.44**	**89.23**	**95.06**	**92.78**

Table 5.8: Comparison of Jackknife accuracy (in percentage) of the best classifier that used the proposed feature and the other reported methods (for 498 Dataset)

Methods	Features used	Jackknife Accuracy				
		All α	All β	$\alpha+\beta$	α/β	Overall
Roughsets	AAC & Physicochemical properties	87.9	91.3	86.0	97.1	90.8
Component coupling	AAC	93.5	88.9	84.5	90.4	89.2
Neural network	AAC	86.0	96.0	86.0	88.2	89.2
SVM	AAC	88.8	95.2	91.5	96.3	93.2
Logitboost	AAC	92.5	96.0	93.0	97.1	94.8
SVM	PSI-BLAST based P-collocated AA pairs	98.0	93.3	93.4	95.6	94.9
SVM Fusion	Pseudo AAC	99.1	96.0	91.5	80.9	91.4
CWT with PCA	Pesudo AAC	94.4	96.8	92.3	97.0	95.2
VPMCD	AAC	93.5	94.3	92.2	97.7	94.5
MODWT with SVM	Physicochemical properties	93.3	94.4	90.7	97.04	93.94
Complexity distance with NN	complexity distance measure	96.3	93.7	89.9	95.6	93.8
GASVM	Physicochemical + structural features	96.3	93.6	89.2	97.8	94.2
Hybrid neural discriminant model	Dipeptide composition frequencies	95.32	88.8	93.02	94.11	92.77
RBFNN	**DCTAmPseAAC**	**95.33**	**95.24**	**94.57**	**98.53**	**96.00**

Chapter 6

An Efficient Hybrid Feature Extraction Method for Classification of Microarray Gene Expression Data

Chapter 6

An Efficient Hybrid Feature Extraction Method for Classification of Microarray Gene Expression Data

6.1 Introduction

The cells are the basic units in Human body which contain identical genetic material. Only a few of these genes are active in every cell which determine the properties of the cell [133]. Evaluation of turned on and turned off states in different cells helps the scientists to understand the normal cell functions and how they are affected when various genes do not perform properly. Many genes are used to specify features unique to each type of cell. For example, Liver cells express genes for enzymes that detoxify poisons, whereas pancreas cells express genes for making insulin. To know how cells achieve such specialization, there is a need to identify the genes expressed by each cell. Molecular biology research evolves through the development of the technologies used for carrying them out. It is not possible to research on a large number of genes using traditional methods. The DNA microarray is one such technologies which helps the researchers to investigate and address issues which were non-traceable earlier. One can analyze the expression of many genes in a single reaction quickly and in an efficient manner. The DNA microarray

technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body. With advanced statistical techniques, microarray analysis enables simultaneous study of the entire genome in a single experiment [134]. It provides substantial effect on tumor diagnosis and classification, prediction of prognosis and response to therapy, and understanding of the molecular mechanisms of tumorigenesis and tumor development. Gene expression profiling by microarray can further refine the future for individualized treatment for cancer patients based on the molecular classification of subtypes.

Microarray technology provides the possibility of creating datasets that capture the information concerning all the relevant genes and proteins for many systems of biological and clinical interest. Such datasets may help scientists to explore gene expression patterns and discover gene interaction networks, and pathways underlying various diseases and biological processes. Such discoveries can in turn lead to better understanding of the physiological functions in healthy and diseased cells, and to diagnose and treat diseases in a better way. Large-scale transcription analyses using microarrays can reveal the molecular mechanisms of physiology and pathogenesis, and therefore can help scientists to develop new diagnostic and therapeutic strategies. Hence there is a need to develop faster, automatic and efficient tools for the analysis of the microarray data.

6.2 Microarray Technology

A microarray is a tool or a laboratory technology for analyzing gene expression that consists of a small membrane or glass slide or silicon chip containing samples of many genes arranged in a regular pattern to test which genes are switched on or off in diseased versus healthy human tissues. A microarray consists of different nucleic acid probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. DNA targets are arrayed onto glass slides

and explored with fluorescent or radioactively labeled probes. The wealth of this kind of data in different stages of cell cycles helps to explore gene interactions and to discover gene functions. The whole process is based on hybridization probing, a technique that uses fluorescently labeled nucleic acid molecules as "mobile probes" to identify complementary molecules, and sequences that are able to base-pair with one another. When a gene is activated, cellular machinery begins to copy certain segments of that gene and produces messenger RNA (mRNA), which is the body's template for creating proteins. The mRNA produced by the cell is complementary, therefore will bind to the original portion of the DNA strand from which it was copied. To determine which genes are turned on and which are turned off in a given cell, first the messenger RNA molecules present in that cell are collected. Then, each mRNA molecule is labeled using a reverse transcriptase enzyme (RT) that generates a complementary cDNA to the mRNA. During that process fluorescent nucleotides are attached to the cDNA. The tumor and the normal samples are labeled with different fluorescent dyes. The labeled cDNAs are placed onto a DNA microarray slide which then hybridized to their synthetic complementary DNAs attached on the microarray slide, leaving its fluorescent tag. Then a special scanner or microscope is used to measure the fluorescent intensity for each spot/areas on the microarray slide. If a particular gene is very active, it produces many molecules of messenger RNA, thus more labeled cDNAs hybridize to the DNA on the microarray slide and generates a very bright fluorescent area. Genes that are less active produce fewer mRNAs, thus less labeled cDNAs, and results in dimmer fluorescent spots. If there is no fluorescence, none of the messenger molecules hybridizes to the DNA, indicating that the gene is inactive. Hence this technique is frequently used to examine the activity of various genes at different times. A pictorial view of the microarray technology is shown in Fig. 6.1. The tumor samples are indicated by red dye and normal samples are marked by green. In the scanned picture of the microarray slide, green represents the Control DNA, which indicates either DNA or cDNA derived from normal tissue and red represents the Sample DNA, which

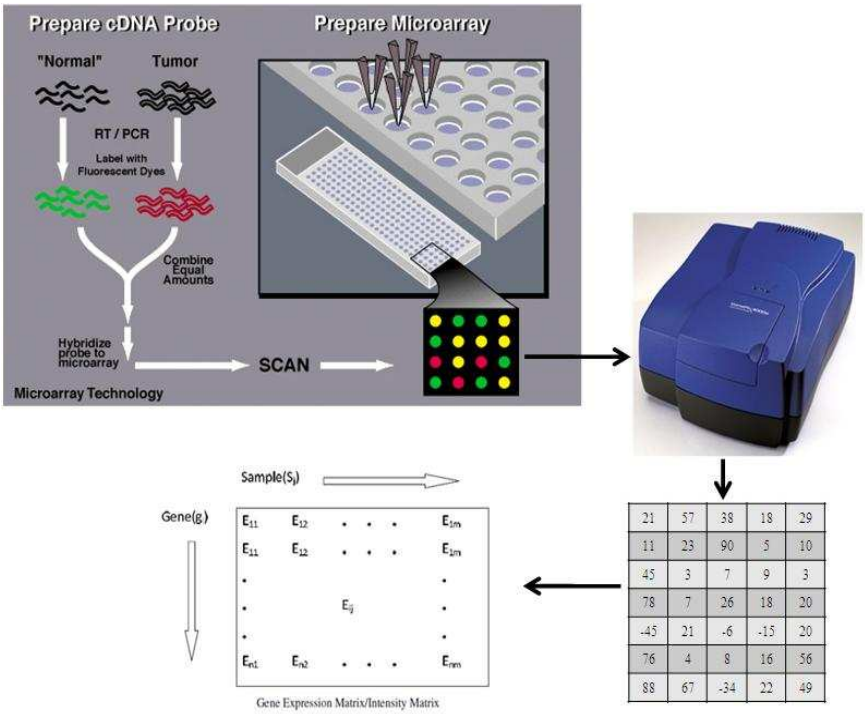

Figure 6.1: The Microarray Techonoly. The tumor and the normal samples are labeled with red and green fluorescent dyes respectively. The labeled cDNAs are placed onto a DNA microarray slide which then hybridized to their synthetic complementary DNAs attached on the microarray slide, leaving its fluorescent tag. Then a scanner is used to measure the fluorescent intensity for each spot/areas on the microarray slide. Each spot on the array is associated with a particular gene and each color in the array represents either healthy or diseased sample. Finally, it produces a gene expression matrix which is a real valued expression level of genes in different samples. In the expression matrix the row contains the expression patterns of the gene, the columns represent the expression profiles of samples.

refers to either DNA or cDNA derived from diseased tissue. The yellow represents a combination of Control and Sample DNA, where both are hybridized equally to the target DNA. Black represent the areas where neither the Control nor Sample DNA hybridized to the target DNA. Each spot on the array is associated with a particular gene and each color in the array represents either healthy (control) or diseased (sample) tissue. Depending on the type of array used, the location and intensity of a color will tell us whether the gene, or mutation, is present in either the control and/or sample DNA. It also provides an estimate of the expression level of the gene(s) in the sample and control DNA. As a result, if the spot is red, this means that specific gene is more expressed in tumor than in normal and if a spot is green, that means that gene is more expressed in the normal tissue. If a spot is yellow that means that that specific gene is equally expressed in normal and tumor tissue.

6.2.1 Gene expression data

Generally a microarray experiment typically assesses a large number of DNA sequences (genes, CDNA clones or expressed sequence tags) under multiple conditions. These conditions may be a time series during a biological process or a collection of different tissue samples (normal versus cancerous tissues). In this chapter, studies have been carried out only on the tissue sample microarray data. Generally a microarray experiment produces a gene expression matrix $E = E_{ij}$, $1 \leq i \leq n, 1 \leq j \leq m$ which is a real valued expression level of genes in different samples as shown in Fig. 6.2. In the expression matrix, the row contains the expression patterns of the gene, the columns represent the expression profiles of samples and each cell is the measured expression level of gene $'i'$ in sample $'j'$.

6.3 Dimension reduction techniques

Dimension reduction is a subject of study in several research areas including high-dimensional data analysis, pattern recognition, and machine learning, where

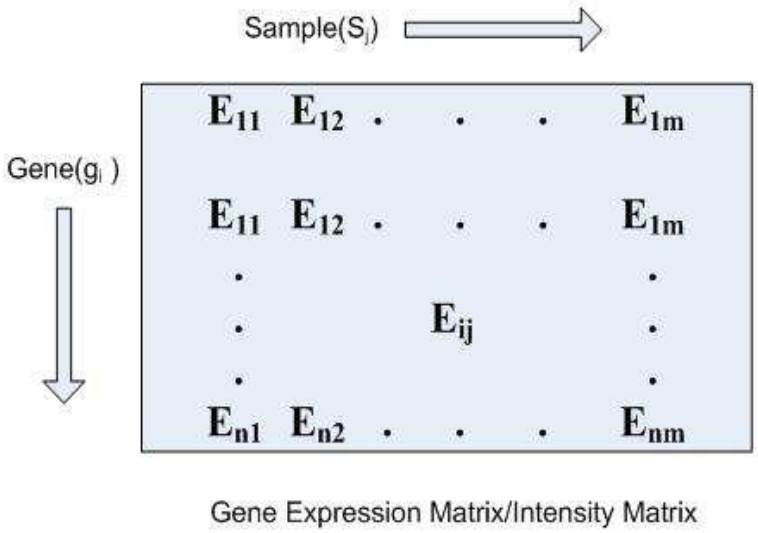

Figure 6.2: The gene expression matrix

one seeks to explain observed high dimensional data using an underlying low-dimensional representation. Dimension reduction has many applications in bioinformatics and computational biology.

Generally the microarray experiments produce large datasets having expression levels of thousands of genes with a very few samples (upto hundreds) i.e high dimensional data. This creates a problem of "curse of dimensionality". Due to this high dimension, the accuracy of the classifier or predictor decreases as it attains the risk of overfitting [135]. As the microarray data contains thousands of genes, a large number of genes are not informative for classification because they are either irrelevant or redundant. Providing a large number of features to learning algorithms can make them inefficient for computational reasons. In addition, irrelevant data may confuse algorithms making them to build inefficient classifiers while correlation between feature sets causes the redundancy of information. Therefore, it is essential to explore the data and utilize independent features to train classifiers. Hence to derive a subset of informative or discriminative genes from the entire gene set is a challenging task in microarray data analysis. The purpose of gene selection or

dimension reduction is to simplify the classifier by retaining small set of relevant genes and to improve the accuracy of the classifier. For this purpose, researchers have applied a number of test statistics or discriminant criteria to find genes that are differentially expressed between the investigated classes.

Dimensionality/feature reduction is essential and can be achieved either by feature selection or transformation to a low dimensional space [136] [143]. The feature selection deals with the reduction of original high dimensional data even further such that the most relevant features are selected for the classification [145]. The methods used for feature selection in the context of microarray data analysis can be broadly categorized into two groups: filter and wrapper approaches [134] [137]. In the filter approach, a selection process precedes the actual classification process where features are evaluated only through intrinsic properties of the data. For each feature a weight value is calculated, and features with better weight values are chosen to represent the original data set. However, the filter approach does not account for interactions between features. The wrapper model approach depends on feature addition or deletion to compose subset features, and uses evaluation function with a learning algorithm to estimate the subset features. This kind of approach is similar to an optimal algorithm that searches for optimal results in a dimension space. The wrapper approach usually conducts a subset search with the optimal algorithm, and a classification algorithm is used to evaluate the subset. Contrary to filter methods, wrapper methods select features specific to the classifier. Hence, they are most likely to be more accurate than filter methods on a particular classifier, but the features they choose may not be appropriate for other classifiers. Another limitation of wrapper methods is that the wrappers are computationally expensive because they need to train and test the classifier for each feature subset candidate, which can be prohibitive when working with high-dimensional data (such as text, image, gene, etc). In contrast to the feature selection, transformation based methods allow modification of the input features to a new feature space. This method deals with the extraction of relevant features by mapping the raw data

onto a lower dimensional space while maintaining the vital information in terms of unique attributes. In feature selection, the original representation of the variables is not changed. Feature selection is typically preferred over transformation when one wishes to keep the original meaning of the features and wishes to determine which of those features are important. Moreover, once features are selected, only these features need to be calculated or collected, whereas, in transformation based methods, all input features are still needed to obtain the reduced dimension.

Various methods and techniques have been developed in recent past to perform the gene selection to reduce the dimensionality problem. The filter method basically use a criterion related to rank and select key genes for classification such as Pearson correlation coefficient method [137], t-statistics method [138], signal-to-noise ratio method [151] and many transformation methods such as the partial least square method, independent component analysis [139], wavelet analysis [141] [142], linear discriminant analysis and principal component analysis [140] have been used to extract the important feature from the microarray data. All the methods transform the original gene space to another domain providing reduced uncorrelated discriminant components. In this chapter, a novel hybrid method has been proposed which combines both the feature selection and extraction method to optimally reflect the characteristics of the microarray data in few feature sets. A F-score statistics is used to preselect the discriminative features from the raw microarray data. Then a model is developed by auto regressive (AR) method to extract the relevant information from gene space.

6.3.1 The F-score method of feature selection

F-score is a popular filter method for gene selection which is based on classical F-statistics, a generalization of T-test for two sample comparison [146] [147]. F-score criterion is the ratio of between class sum of squares to within class sum of squares of individual genes. It is based on statistical F-test and is used for filtering genes with near constant expression across all the samples from important genes. Intuitively,

F-score will be larger for a gene whose expression varies relatively small within a class compared to larger variations between other classes. In this method, a F-score value of each feature in the dataset is computed to show their discriminative power. The F-score value of ith feature of a two class problem is defined as:

$$F(i) = \frac{(\overline{X}_i^{c_1} - \overline{X})^2 + (\overline{X}_i^{c_2} - \overline{X})^2}{\frac{1}{n_{c_1}-1}\sum_{k=1}^{n_{c_1}}(x_{k,i}^{c_1} - \overline{X}_i^{c_1})^2 + \frac{1}{n_{c_2}-1}\sum_{k=1}^{n_{c_2}}(x_{k,i}^{c_2} - \overline{X}_i^{c_2})^2} \tag{6.1}$$

where $\overline{X}, \overline{X}_i^{c_1}, \overline{X}_i^{c_2}$ are the average expression level of gene i across all the samples of c_1 and c_2 classes and $x_{k,i}^{c_1}, x_{k,i}^{c_2}$ are the kth instance of the ith feature for c_1 and c_2 classes.

The numerator of Eq. (6.1) shows the discriminating power between the classes and the denominator reveals that within the individual classes. The larger is the F-score, the more likely the feature is significant in classification. In order to select the efficient features from entire dataset, a threshold value is employed on the F-scores of all features. If the F-score value of any feature is bigger than threshold value, the corresponding feature is added to feature space. Otherwise, it is removed from feature space. Hence, F-score is the ratio of between class sum of squares to within class sum of squares. For any gene, if between class sum of squares is very high for few classes, it may blind the discriminating effect needed for other difficult class prediction. As a result, many genes are selected for such strong class and a very few are top ranked for difficult classes. A disadvantage of F-score is that it does not reveal mutual information among features. Despite of these drawbacks, F-score is simple and generally quite effective in feature selection in high dimensional dataset.

6.3.2 The AR modeling for feature extraction

The autoregressive model is a popular linear model employed for the modeling of time series data generated by a stochastic process in application such as speech processing, image processing, redundancy removal, pattern recognition, etc [41] [42] [148]. It is a simple and robust method and requires no a priori knowledge of the sequence to

be analyzed and also works well with a low signal-to-noise ratio. The parameters of the AR model comprise significant information of the system condition and can reflect the characteristics of a dynamic system. The auto regressive model can be viewed as a linear prediction filter [149]. The coefficients of the linear filter can be used to model the microarray samples in gene space in terms of their global spectral characteristics.

In AR modeling the observed signal $x(n)$ can be modeled as a linear combination of its p past values $x(n-k)$, defined as

$$x(n) = -\sum_{k=1}^{p} a_p x(n-k) \tag{6.2}$$

where a_k represents the coefficient of the model to be estimated. This AR process can be viewed as a recursive all-pole digital filter whose transfer function is given by

$$H(z) = \frac{1}{1 + \sum_{k=1}^{p} a_k z^{-k}} \tag{6.3}$$

The output of the prediction filter is approximately a white noise process if the prediction order is large. Thus, if the filter is inverted and is driven with a white noise sequence, this system could produce a random sequence with same statistical characteristics as that of the original sequence, hence represents a model for the observed signal x(n). The coefficients of the filter can be estimated by the Yule-Walker method in least mean square sense which gives a linear equation defined as

$$\sum_{k=1}^{p} a_k R(i-k) = -R(i), 1 \leq i \leq p \tag{6.4}$$

This can be represented in matrix form as

$$\begin{bmatrix} R_0 & R_1 & R_2 & \cdots & R_{p-1} \\ R_1 & R_0 & R_1 & \cdots & R_{p-2} \\ R_2 & R_2 & R_0 & \cdots & R_{p-3} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ R_{p-1} & R_{p-2} & R_{p-3} & \cdots & R_0 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ \vdots \\ a_p \end{bmatrix} = - \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ \vdots \\ R_p \end{bmatrix} \tag{6.5}$$

where R_p is the autocorrelation of the observed signal and a_p is the model order or parameters of the model. The parameters can be computed by the Levinson Durbin's recursive process. Further details of the AR modeling is available in Ref. [4].

Each microarray sample can be modeled through the autoregressive process to capture its global spectral characteristics. The co-efficients of the model contains the discriminative information regarding the classification of the samples. These co-efficients can be used as the optimal feature set for the microarray samples.

6.4 Classification strategy

Machine learning techniques are increasingly being used to address problems in computational biology and bioinformatics. Novel computational techniques to analyze high throughput data in the form of sequences, gene and protein expressions, pathways, and images are becoming vital for understanding diseases and future drug discovery. In this work, a machine learning technique, the radial basis function network has been used as the classifier to classify the gene expression data. A detailed discussion of RBFNN has been dealt in chapter 2.

6.5 Performance evaluation

To evaluate the classification performance of the proposed hybrid feature vector, a leave one out cross validation (LOOCV) or Jackknife test has been employed on six standard datasets. The Jackknife test is deemed the most objective that can always yield a unique result for a given benchmark dataset, and hence has been widely recognized and increasingly used by investigators to examine the accuracy of various predictors. The generated optimized model has been tested on unseen data to demonstrate the generalization capability of the system. The experiments were conducted using radial basis function network as the basic classifier. The flow chart of the proposed feature extraction scheme along with the classifier is shown in Fig. 6.3.

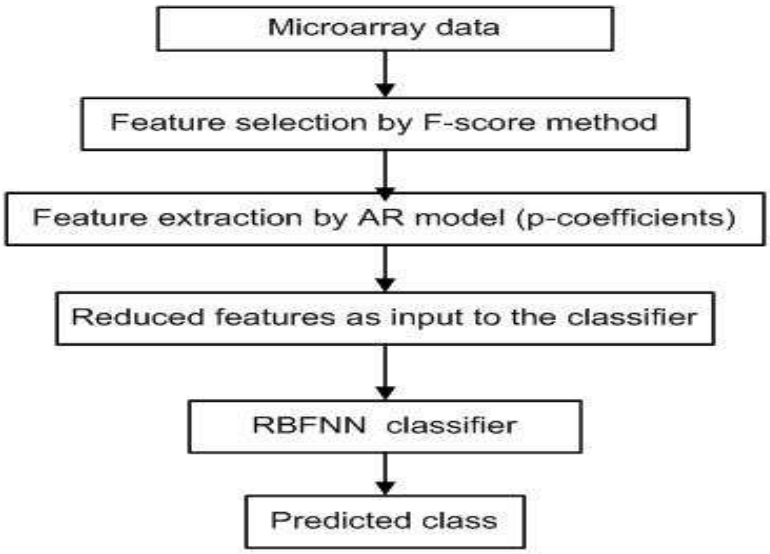

Figure 6.3: The Flow graph of the proposed feature based classification scheme

6.5.1 Datasets

In this section, the cancer gene expression data sets used for the study are described. These datasets are also summarized in Table 6.1.

ALL/AML Leukemia Dataset

This dataset consists of two distinctive acute leukemias, namely AML and ALL bone marrow samples with 7129 probes from 6817 human genes [151]. The training dataset consists of 38 samples (27 ALL and 11 AML) and the test dataset consists of 34 samples (20 ALL and 14 AML).

The dataset is available online at http://www.genome.wi.mit.edu/MPR.

SRBCT Dataset

This dataset consists of four categories of small round blue cell tumors (SRBCT) with 83 samples from 2308 genes [144]. The tumors are Burkitt lymphoma (BL), the Ewing family of tumors (EWS), neuroblastoma (NB) and rhabdomyosarcoma (RMS). There are 63 samples for training and 20 samples for testing. The training

set consists of 8, 23, 12 and 20 samples of BL, EWS, NB and RMS, respectively. The testing set consists of 3, 6, 6 and 5 samples of BL, EWS, NB and RMS, respectively.

The dataset is available online at http://research.nhgri.nih.gov/microarray/Supplements.

MLL Leukemia dataset

This dataset consists of three types of leukemias namely ALL, MLL and AML with 72 samples from 12582 genes [150]. The training dataset consists of 57 samples (20 ALL, 17 MLL and 20 AML) and the test data set consists of 15 samples (4 ALL, 3 MLL and 8 AML).

The dataset is available online at http:// sdmc.lit.org.sg/GEDatasets/.

Prostate Dataset

This dataset consists of prostate tissue samples from 12,600 genes [153]. The training dataset consists of 102 samples out of which 52 are from prostate tumor tissue samples and 50 are from normal tissue sample. The testing dataset consists of 25 tumor samples and 9 normal samples.

The dataset is available online at http:www. Broad.mit.edu/cgi-bin/cancer/data sets.cgi.

Lymphoma Dataset

This data set consists of three most prevalent adult lymphoid malignancies [152]. It consists of 62 samples from 4026 genes. This composes 42, 9 and 11 samples of DLBCL, FL and CL respectively.

The dataset is available online at http://genome-www.stanford.edu/ lymphoma.

Colon Dataset

This dataset consists of total 62 samples collected from colon cancer patients [154]. Among them 40 samples are from tumor tissues and 22 are from healthy parts of the colons of the same patients. The expression levels are measured for 2000 genes.

The dataset is available online at http://microarray.princeton.edu/oncology.

Table 6.1: The standard datasets used for the study

Dataset	number of samples	Classes (number of samples)	number of genes	number of classes
Leukemia	72	ALL (47), AML (25)	7129	2
Colon	62	normal (22), tumor(40)	2000	2
Prostate	102	normal (50), tumor(52)	12,600	2
MLL-Leukemia	72	ALL (24), AML (20), MLL (28)	12582	3
Lymphoma	62	DLBCL (42), FL (9), CL (11)	4026	3
SRBCT	83	BL(29), EWS (11), NB (18), RMS (25)	2308	4

6.5.2 Experimental results

In order to compare the efficiency of the proposed method in predicting the class of the cancer microarray data, six standard datasets such as Leukemia, SRBCT and MLL Leukemia, Colon, Prostate and Lymphoma have been used for the study. All the datasets are categorized into two groups: binary class and multi class to assess the performance of the proposed method. The Leukemia, Prostate and Colon dataset are binary class and SRBCT, Lymphoma and MLL Leukemia are multi class datasets. The feature extraction process proposed in this chapter has two steps. First, the F-score method is employed on gene space to choose the discriminant feature set. For example, The F-scores of the genes in Leukemia dataset is shown in Fig. 6.4.

The average of the F-scores is used as the threshold to select the discriminant genes. Then, the reduced feature set is modeled by the autoregressive modeling to capture the global spectral characteristics of the samples. The parameters of the model contains the information regarding the classification of samples, hence constitutes the optimal features for class prediction. Through an empirical study the model parameters are chosen 50 to achieve better accuracy. The leave one

Table 6.2: Comparison of LOOCV classification accuracy using the proposed feature representation method using RBFNN, MLP and LDA.

Dataset	Method	LOOCV Accuracy
Leukemia	RBFNN	97.22
	MLP	97.22
	LDA	76.39
Colon	RBFNN	93.55
	MLP	90.32
	LDA	64.52
Prostate	RBFNN	93.13
	MLP	88.24
	LDA	71.57
MLL-Leukemia	RBFNN	93.02
	MLP	88.89
	LDA	73.61
Lymphoma	RBFNN	96.77
	MLP	93.55
	LDA	80.65
SRBCT	RBFNN	93.98
	MLP	84.34
	LDA	63.86

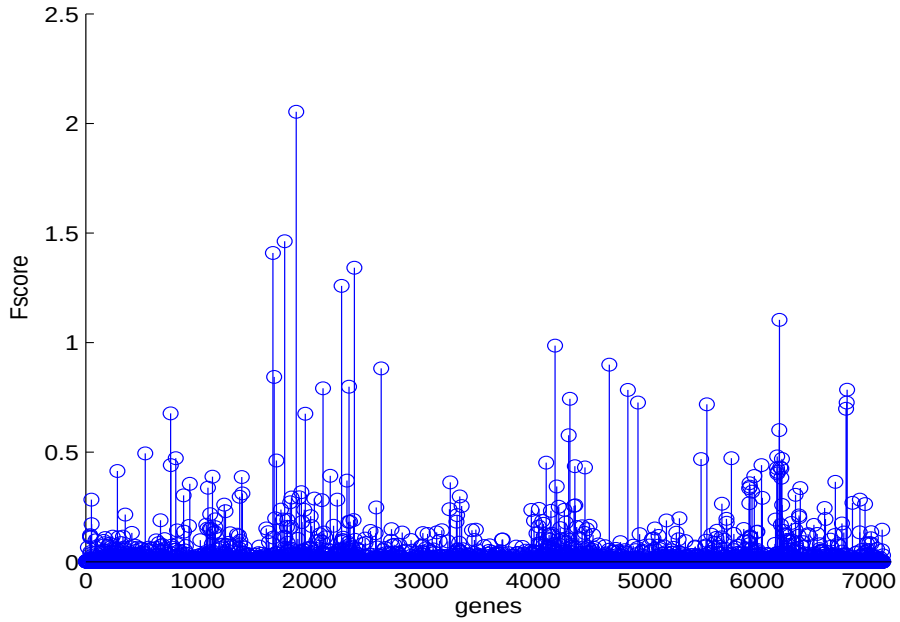

Figure 6.4: The F-score values versus genes of Leukemia dataset.

out cross validation (LOOCV) or jackknife test is conducted by combining all the training and test samples for all the six datasets.

To have a comparative performance study, the proposed feature representation method is analyzed with many well studied classifiers such as neural network (multi layer perceptron), radial basis function network and linear discriminant analysis. The success rates of all the classifiers are evaluated with all the six benchmark datasets. The results of the analysis are listed in Table 6.2 and also presented in histograms in Figs. 6.5 and 6.6. It is clear from the Table that the RBFNN classifier yields best performance among all the classifiers and for all the datasets. Thus the proposed feature extraction method with RBFNN classifier can be effectively used for classification of microarray data.

The performance of the proposed method is also compared with those obtained by the reported best methods in literature and the results are listed in Table 6.3. The existing methods also used the cross validation test on the datasets. From Table 6.3, it reveals that the proposed scheme is equivalent to the counterparts with the

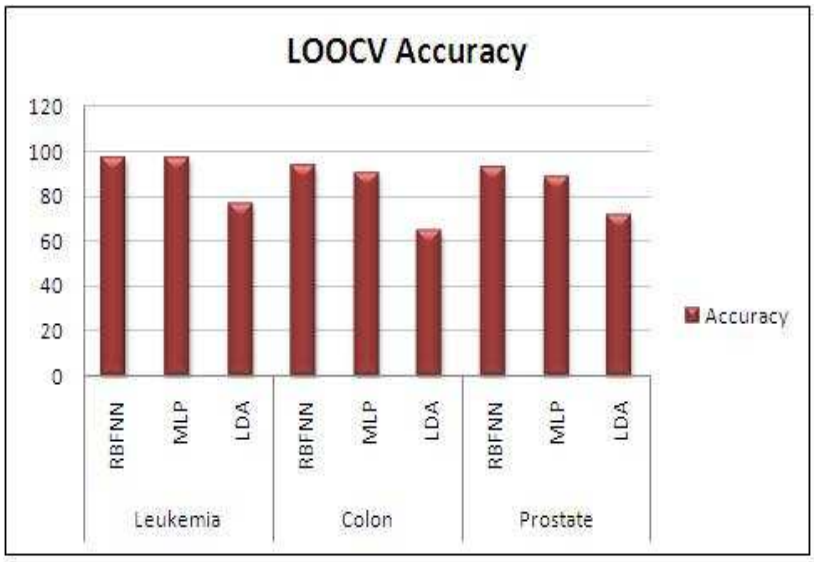

Figure 6.5: The LOOCV accuracy for the binary class datasets (Leukemia, Colon and Prostate).

advantage of reduced computational load.

6.6 Conclusion

In this chapter, an efficient hybrid feature extraction method is presented by embedding the F-score statistics and the AR model. The results of the Jackknife cross validation test using the standard datasets shows that the proposed method can be used as an efficient approach for class prediction of microarray samples. The proposed method employs less features for classification, therefore it involves minimum computational complexity and also the training time of the model is expected to be minimum.

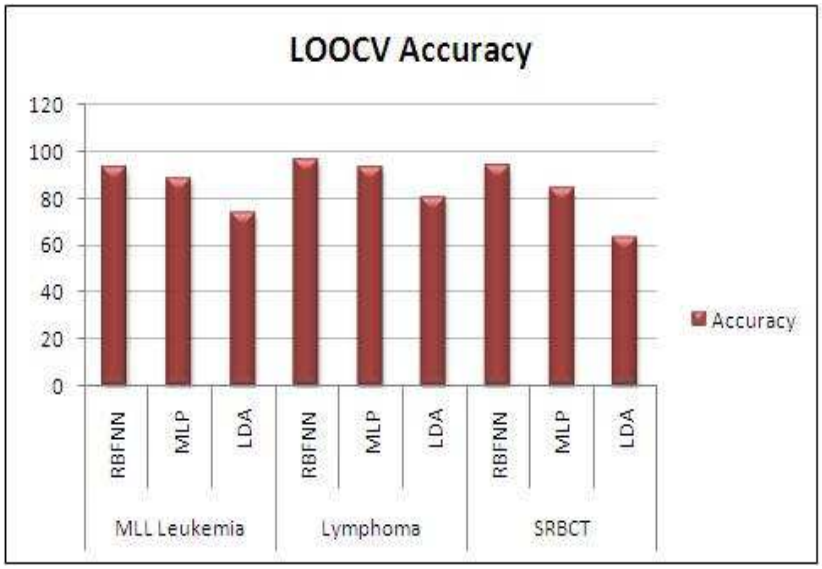

Figure 6.6: The LOOCV accuracy for the multi class datasets (MLL-Leukemia, Lymphoma and SRBCT).

Table 6.3: Comparison of predictive accuracy (%) of the proposed method with the best available method in literature

Dataset	Proposed method	Best available method
Leukemia	97.22	100
Colon	93.55	96
Prostate	93.13	96.08
MLL-Leukemia	93.02	100
Lymphoma	96.77	100
SRBCT	93.98	100

Chapter 7

Conclusion and Future Work

Chapter 7

Conclusion and Future work

7.1 Conclusion

In this chapter, the conclusion of the overall thesis is presented and some of the future research problems which may be attempted by interested readers are outlined. The dissertation has investigated on four important problems in bioinformatics: the protein coding region identification in DNA sequences, hot spot identification in proteins, protein structural class prediction and classification of microarray gene expression data. The novelty of the present work is the introduction of signal processing and machine learning techniques to analyze the genomic and proteomic signals.

Gene prediction and protein coding region identification in DNA sequences are unsolved and popular research problems in bioinformatics. Several powerful computational methods have been developed for coding region identification and their performance is highly dependent on the coding measures that are used for characterizing DNA sequences. The period-3 property exhibited by the coding regions is used as the basis to identify them in the sequence. In this dissertation a new time-frequency filtering approach based on S-transform technique is presented to efficiently detect the protein coding regions. First, the spectrum of the DNA sequence is computed using S-transform to localize the period-3 frequency in time-frequency plane. Then, that pattern is filtered out using a mask in the

time-frequency domain, thereby producing peaks in the energy sequence wherever the coding regions present. The potential of the proposed method is assessed by evaluating ROC curves and statistical parameters (Sp, Sn, Average accuracy) in C. elegans chromosome and HRM dataset. The results obtained by the proposed method are also compared with the conventional sliding window DFT and anti-notch filtering method. The comparison study reveals that the proposed approach provides an average accuracy of 96% in gene F56F11.4 of C. elegans chromosome III.

Proteins are the biomolecules that govern most of the functions in the living cells. The function of protein is dependent on the 3-D structure which facilitates the interactions of protein with other molecules at a specific site known as active sites. Some residues in these sites, known as hot spots are responsible for the binding. Hence, the identification of these hot spots in protein is a challenging issue in protein science. In this dissertation a novel S-transform based filtering approach is proposed to identify these hot spots. It is a sequence based approach based on the concept of RRM. The characteristic frequency that corresponds to the interaction of proteins is first localized in the time-frequency spectrum and then filtered out from the sequence to provide a measure to identify the hot spots. The performance of the proposed method is compared with the corresponding results obtained by the existing computational methods in terms of sensitivity, specificity, positive predictive value, negative predictive value and average accuracy in some standard examples of proteins. A comparison study reveals the potentiality of the proposed method. It also identifies some more unknown hot spot locations in the proteins which need further investigation to include in the database for future reference.

The structural class is one of the most important features for characterizing the overall folding type of a protein. Thus prediction of structural class of protein is necessary and has been a challenging problem in bioinformatics. This dissertation work presents a novel feature representation scheme (DCTAmPseAAC) based on the Chou's pseudo amino acid composition for efficient prediction of the structural classes of proteins. It efficiently captures the characteristics of protein relevant to

structure, thereby improves the performance of the classifier. The potential of the proposed method is assessed by the Jackknife test on three benchmark datasets: 204, 277 and 498 datasets. A simple radial basis function network is introduced for an efficient classification of the proteins into different structural classes. An exhaustive simulation study of the proposed scheme demonstrates its superiority by providing at least 2-3% improvement in the prediction accuracy over the existing methods.

Microarray technology allows scientists to measure the expression levels of thousands of genes simultaneously from a single experiment and thereby facilitates the understanding of the molecular interaction and functions. It provides a way to characterize the state of cells or tissues and to associate that state with a phenotypic trait. The outcomes from microarray technology are used to provide a fundamental understanding of biological development as well as to explore the underlying genetic causes of many biological functions such as disease. In this context cancer classification is important for subsequent diagnosis and treatment. The high dimension and low sample size of microarray data lead to curse of dimensionality problem which affects the classification performance. In this dissertation an efficient hybrid feature extraction method is proposed to overcome the dimensionality problem. First, the discriminative genes are selected by the F-score statistics and then, the reduced feature set is used by the autoregressive model to efficiently characterize the samples. Exhaustive simulation study is carried out on the proposed method with six standard cancer microarray datasets to demonstrate its potential.The results show that the proposed method is comparable to the existing best algorithm but with an advantage of reduced complexity.

The objectives of the dissertation proposed in chapter 1 have been met in introducing novel signal processing methods to compute the measures or extract features by demonstrating the efficacies of these methods with soft computing techniques.

7.2 Future work

The work carried out in the present dissertation can be extended in many directions.

- The time-frequency resolution of S-transform can further be improved by efficiently scaling the Gaussian window and the filtering capability can be improved by efficiently designing the time-frequency filter. Use of such efficient filter is expected to achieve improved performance in identification tasks.

- In the dissertation a new feature representation that is DCTAmPseAAC has been suggested.The use of such features has resulted in improved classification performance. It is proposed that the new feature representation can be employed for efficient identification of protein membrane type, enzyme family classification and many such applications.

- In recent years many evolutionary computing algorithms such as genetic algorithm (GA), differential evolution (DE), particle swarm optimization (PSO) and artificial immune system (AIS) have been developed and applied to many fields. In the field of bioinformatics these new optimization techniques have not been gainfully applied. Hence there are wide scopes of applying these emerging tools for achieving potential solution of the various bioinformatics problems attempted in this dissertation.

- Further many bioinformatics problems involving pattern identification and feature extraction can be formulated or viewed as multiobjective optimization problems. After such formulation multiobjective optimization algorithms such as NSGA-II, MPSO and MBFO can be conveniently applied to obtain improved solutions.

Annexure-I

Figure 7.1: The EIIP coded sequence (1000 bases) of the gene F56F11.4 [87]

Annexure-II

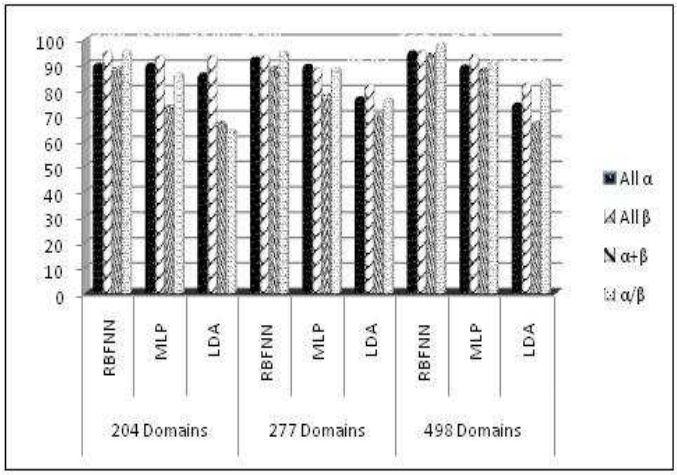

Figure 7.2: Comparison of Jackknife accuracies of all classes of different classification algorithms using the proposed DCTAmPseAAC feature representation method

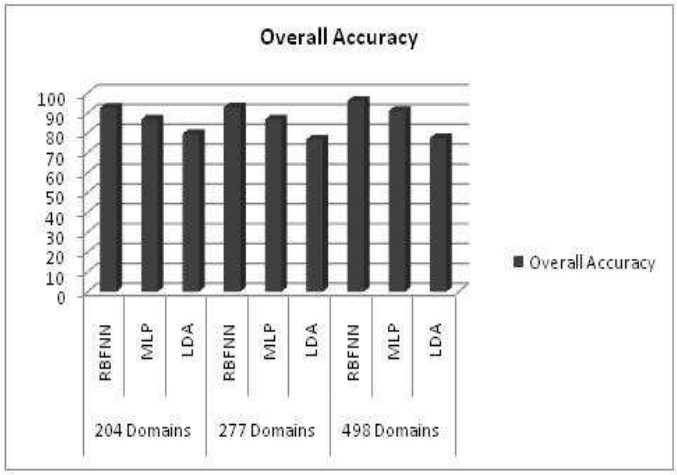

Figure 7.3: Comparison of overall Jackknife accuracies of different classification algorithms using the proposed DCTAmPseAAC feature representation method

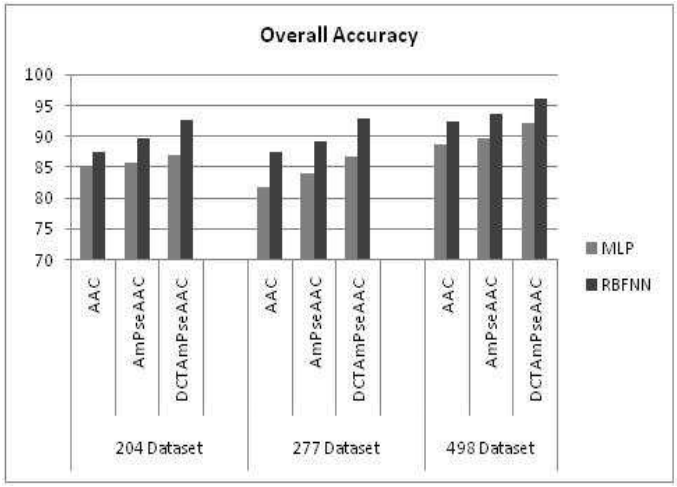

Figure 7.4: Comparison of overall Jackknife classification accuracy with the three feature representations using RBFNN and MLP

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Dissemination of Work

Journals

1. **Sitanshu Sekhar Sahu** and Ganapati Panda. Efficient Localization of Hot Spot in Proteins Using A Novel S-Transform Based Filtering Approach. *IEEE/ACM Transaction on Computational Biology and Bioinformatics*, vol. 8, issues 5, pp. 1235-1246, 2011.

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3. **Sitanshu Sekhar Sahu** and Ganapati Panda. A Novel Feature Representation Method Based on Chou's Pseudo Amino Acid Composition for Protein Structural Class Prediction. *Journal of Computational Biology and Chemistry,Elsevier*, vol. 34, issues 5-6, pp. 320-327, 2010.

Conferences

1. **Sitanshu Sekhar Sahu** and Ganapati Panda. A New Approach for Identification of Hot Spots in Proteins Using S-Transform Filtering. In *IEEE International Workshop on Genomic Signal Processing and Statistics (GENSIPS)*. pp. 1–4, Minnesota, USA, 2009.

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4. **Sitanshu Sekhar Sahu**, Ganapati Panda and Ramchandra Barik. Cancer Classification Using Microarray Gene Expression Data: Approached Using Wavelet Transform and F-score Method. In *International Conference on Electronic Systems(ICES)*, pp. 42–45, NIT, Rourkela,India, 2011.

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